EXHIBIT A

US007449464B2

(12) **United States Patent**
Martin et al.

(10) **Patent No.: US 7,449,464 B2**
(45) **Date of Patent: Nov. 11, 2008**

(54) **PHTHALAZINONE DERIVATIVES**

(75) Inventors: **Niall Morrison Barr Martin**, Cambridge (GB); **Graeme Cameron Smith**, Cambridge (GB); **Stephen Philip Jackson**, Cambridge (GB); **Vincent Junior M Loh**, Horsham (GB); **Xiao-Ling Fan Cockcroft**, Horsham (GB); **Ian Timothy Williams Matthews**, Horsham (GB); **Keith Allan Menear**, Horsham (GB); **Frank Kerrigan**, Cornwall (GB); **Alan Ashworth**, London (GB)

(73) Assignees: **Kudos Pharmaceuticals Limited**, Cambridge (GB); **Maybridge Limited**, Cornwall (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 213 days.

(21) Appl. No.: **10/876,080**

(22) Filed: **Jun. 24, 2004**

(65) **Prior Publication Data**

US 2005/0059663 A1 Mar. 17, 2005

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/799,154, filed on Mar. 12, 2004, now abandoned.

(60) Provisional application No. 60/454,995, filed on Mar. 14, 2003, provisional application No. 60/526,244, filed on Dec. 1, 2003, provisional application No. 60/493,399, filed on Aug. 6, 2003.

(30) **Foreign Application Priority Data**

Mar. 12, 2003 (GB) 0305681.9

(51) **Int. Cl.**
A01N 43/60 (2006.01)
A01N 43/58 (2006.01)
A61K 31/50 (2006.01)
A61K 31/495 (2006.01)
C07D 487/00 (2006.01)

(52) **U.S. Cl.** **514/248**; 514/236; 514/237; 544/236; 544/237

(58) **Field of Classification Search** 514/248, 514/236, 237; 544/236, 237
See application file for complete search history.

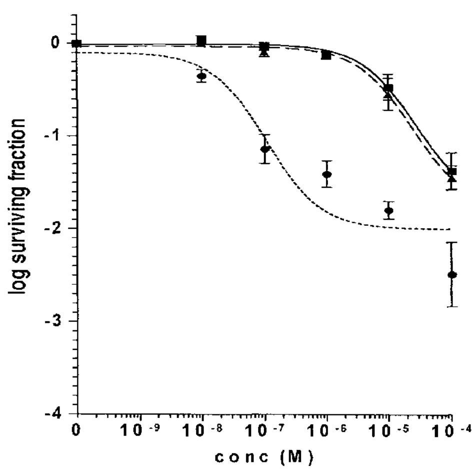
(56) **References Cited**

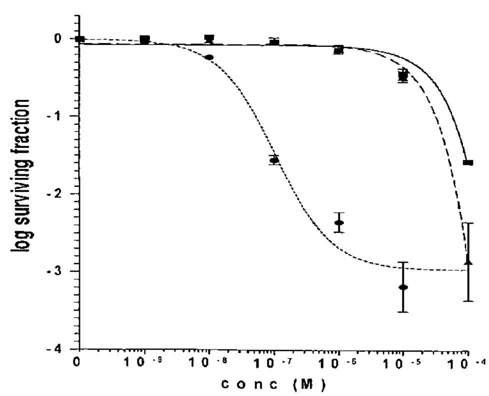
U.S. PATENT DOCUMENTS

3,813,384	A	5/1974	Vogelsang et al.
4,665,181	A	5/1987	Thomas et al.
4,841,047	A	6/1989	Engel et al.
5,032,617	A	7/1991	Lee et al.
5,041,653	A	8/1991	Lee et al.
5,215,738	A	6/1993	Lee et al.
5,556,856	A	9/1996	Engel et al.
5,587,384	A	12/1996	Zhang et al.
5,648,355	A	7/1997	Theoharides
5,874,444	A	2/1999	West
5,886,178	A	3/1999	Allen et al.
6,197,785	B1	3/2001	Jackson et al.
6,340,684	B1	1/2002	Napoletano et al.
6,426,415	B1	7/2002	Jackson et al.
6,476,048	B1	11/2002	Szabo et al.
6,498,160	B2	12/2002	Napoletano et al.
6,514,983	B1	2/2003	Li
6,514,984	B1	2/2003	Watanabe
6,635,642	B1	10/2003	Jackson et al.
6,677,333	B1	1/2004	Seko et al.
2002/0183325	A1	12/2002	Martin et al.
2004/0023968	A1	2/2004	Martin et al.
2005/0059663	A1	3/2005	Martin et al.
2005/0080096	A1	4/2005	Ishida et al.
2005/0227919	A1	10/2005	Kudos

FOREIGN PATENT DOCUMENTS

DE	2143745	3/1973
DE	3813531	4/1988
DE	287 032	2/1991
EP	0030861	6/1981
EP	0269968	6/1988
EP	0 355 750	2/1990
EP	0389995	10/1990
EP	0502575	9/1992





EP	0 590 551		4/1994
EP	0634404	A1	1/1995
EP	699754		6/1996
EP	705903		10/1996
EP	0 792 643		9/1997
FR	2 262 513		9/1975
GB	721286		1/1955
GB	2 384 776		3/2004
IT	MI98A001671		4/1999
JP	54156526		12/1979
JP	58164577		9/1983
JP	62-252774		11/1987
WO	WO 91/18591		12/1991
WO	WO 93/14086		7/1993
WO	WO 94/10151		5/1994
WO	WO 95/24379		9/1995
WO	WO 96/19225		6/1996
WO	WO 98/43477		10/1998
WO	WO 98/51308		11/1998
WO	WO 99/08680		2/1999
WO	WO 99/11624		3/1999
WO	WO 99/11645		3/1999
WO	WO 99/11649		3/1999
WO	WO 99/44612		9/1999
WO	WO 99/47494		9/1999
WO	WO 00/05219		2/2000
WO	WO 00/42040		7/2000
WO	WO 00/44726		8/2000
WO	WO 00/67734		11/2000
WO	WO 01/12199		2/2001
WO	WO 01/16136		3/2001
WO	WO 01/16137		3/2001
WO	WO 01/21615		3/2001
WO	WO 01/23390		4/2001
WO	WO 01/57038		8/2001
WO	WO 01/79184		10/2001
WO	WO 01/85686		11/2001
WO	WO 01/85687		11/2001
WO	WO 01/87845		11/2001
WO	WO 01/90077		11/2001
WO	WO 02/36576		5/2002
WO	WO 03/070726		5/2002
WO	WO 02/44157		6/2002
WO	WO 02/068407		9/2002
WO	WO 02/090334		11/2002
WO	WO 02/094790		11/2002
WO	WO 03/007959		1/2003
WO	WO 03/051879		6/2003
WO	WO 03/055865		7/2003
WO	WO 03/057145		7/2003
WO	WO 03/063874		8/2003
WO	WO 03/070707		8/2003
WO	WO 03/080581		10/2003
WO	WO 03/093261		11/2003
WO	WO 2004/080976		9/2004
WO	WO 2005/053662		6/2005

OTHER PUBLICATIONS

Vippagunta et al., "Crystalline solids", Advanced Drug Delivery Reviews 48 (2001)3-26.*

U.S. Appl. No. 11/873,671, filed Oct. 17, 2007, entitled "Phthalazinone Derivative."

"Applied Biosystems 430A Users Manual", ABI Inc., Foster City, California (book not provided).

"Current Protocols in Molecular Biology", 1992, Eds. AUSUBEL et al., John Wiley & Sons (book not provided).

"From DNA damage and stress signalling to cell death", 2000, Eds. De Murcia, G and Shall, S, Oxford University Press (book not provided).

"Handbook of Pharmaceutical Additives", 2001, 2 Ed., Synapse Information Resources Inc., Endicott, New York, USA (book not provided).

"Handbook of Pharmaceutical Excipients", 1994, 2 Ed. (book not provided).

"Molecular Cloning: A Laboratory Manual", 2001, 3 Ed., Sambrook & Russell, Cold Spring Harbor Laboratory Press, New York (book not provided).

"PCR Protocols; A Guide to Methods and Application", 1990, Eds. Innis et al., Academic Press, New York (book not provided).

"PCR Technology", 1989, Eds. EHRLICH, Stockholm Press, New York (book not provided).

"Remington's Pharmaceutical Sciences", 2000, 20 Ed., Lippincott, Williams & Wilkins (book not provided).

Affar, E. B., et al., *Anal. Biochem.*, 1998, vol. 259, No. 2, pp. 280-283.

Al-Dabbagh and Smith, "Species differences in oxidative drug metabolism: some basic considerations." Archives of toxicology, Supplement. Archiv fur Toxikologie. Supplement, vol. 7, pp. 219-231 (1984).

Althaus, F.R. and Richter, C., 1987, ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin (book not provided).

Ame, et al., *J. Biol. Chem.*, 1999, vol. 274, p. 17860-17868.

Angell, et al., *EMBO J.*, 1997, vol. 16, No. 12, pp. 3675-3684.

Arnaudeau, C., et al., *J. Mol. Biol*, 2001, vol. 307, pp. 1235-1245.

Banasik, M., et al., "Specific Inhibitors of Poly (ADP-Ribose) Synthetase and Mono (ADP-ribosyl) transferse", *J. Biol. Chem.*, 1992, vol. 267, pp. 1569-1575.

Banasik, M., et al., *Mol. Cell Biochem.*, 1994, vol. 138, pp. 185-197.

Ben-Hur et al., 1984, *British Journal of Cancer*, 49 (Suppl. VI): 39-42.

Berge et al., 1977, "Pharmaceutically Acceptable Salts," *J. Pharm. Sci.*, vol. 66, pp. 1-19. *Protective Groups in Organic Synthesis* (T. Green and P. Wuts, Wiley, 1991).

Berger, N. A., et al., "Poly (ADP-ribose) in cellular response to DNA damage", *Radiation Research*, 1985, vol. 101, pp. 4-14.

Bhattacharyya, A., et al., *J. Biol. Chem.*, 2000, vol. 275, pp. 23899-23903.

Bodanzsky, M., Bodanzsky, A., "The Practice of Peptide Synthesis", 1984, Springer Verlag, New York (book not provided).

Bold et. al., J. Med. Chem., 2000, 43, 3200.

Bold, G., et al., "New anilinophthalazines as potent and orally well absorbed inhibitors of the FEBF receptor tyrosine kinases useful as antagonists of tumour-driven angiogenesis", *J. Med. Chem.*, 2000, vol. 43, No. 12, pp. 2310-2323.

Bowman et al., "Differential effects of the poly (ADP-ribose) polymerase (PARP) inhibitor NU 1025 on topoisomerase I and II inhibitor cytotoxicity in L1210 cells in vitro "Britishi Journal of Cancer, vol. 84(1), pp. 106-112 (2001).

Brummelkamp, T. R., et al., *Science*, 2002, vol. 296, pp. 550-553.

Bundgaard, Hans, Design of Prodrugs, p. 1 © 1985 Elsevier Science Publishers.

Burzio, L., et al., "Poly (adenosine diphosphoribose) synthase activity of isolated nuclei of normal and leukemic leukocytes (38930)", *Proc. Soc. Exp. Bio. Med.*, 1975, vol. 149, pp. 933-938.

Cantoni, O., et al., "Hydrogen peroxide insult in cultured mammalian cells: relationships between DNA single-strand breakage, poly (ADP-ribose) metabolism and cell killing", *Biochim. Biophys. Acta*, 1989, vol. 1014, 1-7.

Catteau, A., et al., *Oncogene*, 1999, vol. 18, pp. 1957-1965.

Chappuis, P. O., et al., *Cancer Treat. Res.*, 2002, vol. 107, pp. 29-59.

Cosi, C., et al., "Poly (ADP-ribose) polymerase: early involvement in glutamate-induced neurotoxicity in cultured cerebellar granule cells", *J.Neurosci. Res.*, 1994, vol. 39, pp. 38-46.

Cosi, Cristina, "New inhibitors of poly(ADP-ribose) polymerase and their potential therapeutic targets," Expert Opin. Ther. Patents (2002) 12(7): 1047-1071.

Couzin, J., *Science*, 2003, vol. 302, pp. 591-592.

Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 1992, vol. 32, pp. 329-376.

D'Adda Di Fagagna, F., et al., "Functions of poly(ADP-ribose) polymerase in controlling telomere length and chromosomal stability", *Nature Gen.*, 1999, vol. 23, No. 1, pp. 76-80.

D'Amours, D., et al., "Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions", *Biochem. J.*, 1999, vol. 342, pp. 249-268.

D'Andrea, A. D., et al., *Nat. Rev. Cancer*, 2003, vol. 3, pp. 23-34.

Dantzer, F., et al., *Biochemistry*, 2000, vol. 39, pp. 7559-7569.

Davies, A. A., et al., *Mol. Cell*, 2001, vol. 7, pp. 273-282.
Dillon, K. J., et al., *J. Biomolecular Screening*, 2003, vol. 8, No. 3, pp. 347-352.
Durkacz, B. W., et al., "(ADP-ribose)$_n$ participates in DNA excision repair", *Nature*, 1980, vol. 283, No. 7, pp. 593-596.
Ehrlich, et al., *Science*, 1991, vol. 252, pp. 1643-1650.
Elbashir, S. M., et al., *Nature*, 2001, vol. 411, pp. 494-498.
El-Tamaty , et al., Synthesis and biological activity of some 4-benzyl-1(2H)-phthalazinone derivatives, *Indian J. Chemistry*, v. 35B, pp. 1067-1072 (Oct. 1996).
Esteller, M., et al., *J. Natl. Cancer Inst.*, 2000, vol. 92, pp. 564-569.
Fire, A., et al., *Nature*, 1998, 391: 806-811.
Foray, et al., *Embo J.*, 2003, vol. 22, pp. 2860-2871.
Fujisawa Pharm., Chemical Abstracts, vol. 109:6531, 1988.
Fuska, J., et al., "New Cytotoxic and antitumor agents," Chemical Abstracts, 104:102050 (1986).
Gaken, J. A., et al., "Efficient retroviral infection of mammalian cells is blocked by inhibition of poly(ADP-ribose) polymerase activity", *J. Virology*, 1996, vol. 70, No. 6, pp. 3992-4000.
Green, T. et al., "Protective Groups in Organic Synthesis", 1999, 3 Ed., Wiley (book not provided).
Griffin et al., "The role of inhibitors of poly (ADP-ribose) polymerase as resistance-modifying agents in cancer therapy" Biochim vol. 77, pp. 408-422 (1995).
Haber, J. E., *Trends Biochem. Sci.*, 1999, vol. 24, pp. 271-275.
Hall, Iris H., et al., "Cytotoxicity of imides-N-alkyl semicarbazones, thiosemicarbazones, acetylhydrazones and related derivatives," *Anti-Cancer Drugs* (and abstract 122:204573), v.6, 147-153 (1995).
Hawley's Condensed Chemical Dictionary, 13th ed. pp. 716 and 825 © 1997 by Van Nostrand Reinhold.
Hirai, K., et al., "Aberration of poly(adenosine diphosphate-ribose) metabolism in human colon adenomatous polyps and cancers", *Cancer Res.*, 1983, vol. 43, pp. 3441-3446.
Hiramoto, T., et al., *Oncogene*, 1999, vol. 18, pp. 3422-3426.
Hoeijmakers, J. H., *Nature*, 2001, vol. 411, pp. 366-374.
Hughes-Davies, et al., *Cell*, vol. 115, pp. 523-535.
Islam et al., Chemical Abstracts, vol. 87:67943, 1977.
Islam et al., Chemical Abstracts, vol. 95:187182, 1981.
Islam et al., Chemical Abstracts, vol. 95:62106, 1981.
Janatova, M., et al., *Neoplasma*, 2003, vol. 50, No. 4, pp. 246-250.
Jancarkova, N., *Ceska Gynekol.*, 2003, vol. 68, No. 1, pp. 11-16.
Jasin, M., *Oncogene*, 2002, vol. 21, No. 58, pp. 8981-8993.
Kashani-Sabet, et al., *Cancer Gene Therapy*, 1995, vol. 2, No. 3, pp. 213-223.
Kawamura, Ikuo, et al., "Ponalrestat, an aldose reductase inhibitor," Chemical Abstract 132:273943.
Kerr, P., et al., *Curr. Biol.*, 2001, vol. 11, pp. R668-R676.
Kerrigan, F., et al., Poster at 12th SCI-RSC medicinal Chemistry Symposium, Cambridge, Sep. 7-10, 2003.
Khanna, K. K., et al., *Nat. Genet.*, 2001, vol. 27, No. 3, pp. 247-254.
Kraakman-Van Der Zwet, M., et al., *Mol. Cell Biol.*, 2002, vol. 22, pp. 669-679.
Lakhani, S. R., et al., *J. Clin. Oncol.*, 2002, vol. 20, pp. 2310-2318.
Le Rhun, Y., et al., "Cellular responses to DNA damage in the absence of poly(ADP-ribose)polymerase", *Biochem. Biophys. Res. Commun.*, 1998, vol. 245, pp. 1-10.
Lemay, M., et al., *Biotechniques*, 1999, vol. 27, pp. 846-851.
Liaudet, L., et al., "Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose)polymerase", *Proc. Natl. Acad. Sci. U.S.A.*, 2000, vol. 97, No. 3, pp. 10203-10208.
Martin, N., et al., "Phthalazinone derivatives as potent PARP-I inhibitors", *13th Intl. Symposium on ADP-ribosylation*, 2001, Abstract 107.
Martin, N., et al., J. Photochem. and PhotoBiol. B: Biology, 2001, vol. 63, pp. 162-170.
Matsuda, M., et al., *Oncogene*,, 1999, vol. 18, pp. 3427-3430.
Menissier de Murcia, J. et al., "Requirement of poly(ADP-ribose)polymerase in recovery from DNA damage in mice and cells", *Proc. Natl. Acad. Sci. U.S.A.*, 1997, vol. 94, pp. 7303-7307.
Mercola, et al., *Cancer Gene Therapy*, 1995, vol. 2, No. 1, pp. 47-59.
Miwa, M., et al., "Cell density-dependent increase in chromatin-associated ADP-ribosyltransferase activity in simian virus 40-transformed cells", *Arch. Biochem. Biophys.*, 1977, vol. 181, pp. 313-321.
Moynahan, M. E., et al., *Mol. Cell*, 1999, vol. 4, pp. 511-508.
Moynahan, M. E., et al., *Mol. Cell*, 2001, vol. 7, pp. 263-272.
Mullis, et al., *Cold Spring Harbor Symp. Quant. Biol.*, 1987, vol. 51, pp. 263-273.
Nathanson, K. L., et al., *Nat. Med.*, 2001, vol. 7, pp. 552-556.
Neuhausen, S. L., et al., *Genet. Test*, 1997, vol. 1, pp. 75-83.
Noel, G., et al., *BMC Cell Biol.*, 2003, vol. 4, pp. 7.
Pacher, et al., "The Role of Poly(ADP-Ribose) Polymerase Activation in the Development of Myocardial and Endothelial Dysfunction in Diabetes" Diabetes, Feb. 2002, vol. 51, 514-521.
Perkins, E., et al., "Novel inhibitors of poly(ADP-ribose)polymerase/PARP1 and PARP2 identified using a cell-based screen in yeast", *Cancer Res.*, 2001, vol. 61, pp. 4175-4183.
Radice, P. J., *Exp. Clin. Cancer Res.*, 2002, vol. 21 (3 Suppl.), pp. 9-12.
Rattan, S. I., et al., "Kinetin delays the onset of ageing characteristics in human fibroblasts", *Biochem. Biophys. Res. Commun.*, 1994, vol. 201, No. 2, pp. 665-672.
Said, S. I., et al., "Excitotoxicity in the lung: N-methy-D-aspartate-induced, nitric oxide-dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose)polymerase", *Proc. Natl. Acad. Sci. U.S.A.*, 1996, vol. 93, pp. 4688-4692.
Schlicker, A., et al., "4-Amino-1,8-napthalimide: a novel inhibitor of poly(ADP-ribose)polymerase and radiation sensitizer", *Int. J. Radiat. Bio.*, 1999, vol. 75, No. 1, pp. 91-100.
Schultz, N., et al., *Nucleic Acids Res.*, 2003, vol. 31, pp. 4959-4964.
Shall, S., et al., *Mutat. Res.*, 2000, vol. 460, pp. 1-15.
Shimizu, T. et al., "Inhibitory effects of azelastine and tranilast on leukotriene B_4 and leukotriene C_4 generation by rat colonic mucosa", *Prostaglandins Leukotrienes and Essential Fatty Acids*, 1995, vol. 53, pp. 355-358.
Silverman, Richard B., *The Organic Chemistry of Drug Design and Drug Action*, pp. 352-400 © 1992 Academic Press, Inc.
Skehan, P., et al., "New colorimetric cytotoxicity assay for anticancer-drug screening", *J. Natl. Cancer Inst.*, 1990, vol. 82, No. 13, pp. 1107-1112.
Southan, Garry J. and Szabo, Csaba "Poly (ADP-ribose) polymerase inhibitors," *Current Medicinal Chemistry*, 10:4, 321-340 (2003).
Stewart, J. M., Young, J. D., "Solid Phase Peptide Synthesis", 1984, 2 Ed., Pierce Chemical Company, Rockford, Illinois (book not provided).
Suto, et al., *Anticancer Drug Des.*, 1991, vol. 6, pp. 107-117.
Szabo, C., et al., "Endothelial dysfunction in a rat model of endotoxic shock", *J. Clin. Invest.*, 1997, vol. 100, pp. 723-725.
Taniguchi, T., et al., *Nat. Med.*, 2003, vol. 9, pp. 568-574.
Tarsounas, M., et al., *Oncogene*, 2003, vol. 22, pp. 1115-1123.
Thompson, L. H., et al., *Mutat. Res.*, 2002, vol. 509, pp. 49-78.
Tracey, W., et al., "Aldose reductase inhibition alone or combined with an adenosine A3 agonist reduces ischemic myocardial injury," Chemical Abstract 134:65983.
Tutt, et al., *EMBO J.*, 2001, vol. 20, pp. 4704-4716.
Tutt, et al., *EMBO Rep.*, 2002, vol. 3, pp. 255-260.
Tutt, et al., *Trends Mol. Med.*, 2002, vol. 8, No. 12, pp. 571-576.
Uhlmann, E. and Peyman, A. et al., *Chem. Rev.*, 1990, vol. 90, pp. 543-584.
Venkitaraman, A. R., *Cell*, 2002, vol. 108, pp. 171-182.
Virag and Szabo, "The Therapeutic Potential of Poly(ADP-Ribose) Polymerase Inhibitors" Pharmacological Reviews, vol. 54(3) pp. 375-429 (2002).
Voinnet, et al., *Nature*, 1997, vol. 389, pp. 553.
Wang, Z.-Q. et al., "Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease", *Genes Dev.*, 1995, vol. 9, pp. 509-520.
Wang, Z.-Q. et al., *Genes Dev.*, 1997, vol. 11, pp. 2347-2358.
West, Anthony R. "Solid State Chemistry and its Applications" Wiley, New York, pp. 358 and 365 (1988).
Wood, et al., *Science*, 2001, vol. 291, pp. 1284-1289.
Yamaguchi, M., et al., "Novel antiasthmatic agents with dual activities of thromboxane A_2 synthetase inhibition and bronchodilation. 1. 2-[2-(1-Imidazolyl)alkyl]-1(2*H*)-phthalazinones", *J. Med. Chem.*, 1993, vol. 36, No. 25, pp. 4052-4060.

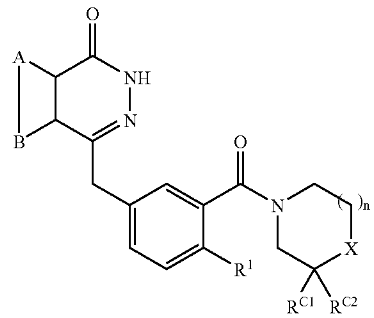
Yamaguchi, M., et al., "Novel antiasthmatic agents with dual activities of thromboxane A_2 synthetase inhibition and bronchodilation. 2. 4-(3-Pyridyl)-1(2*H*)-phthalazinones", *J. Med. Chem.*, 1993, vol. 36, No. 25, pp. 4061-4068.
Zamore, P. D., *Cell*, 2000, vol. 101, pp. 25-33.
Zamore, P. D., *Nature Structural Biology*, 2001, vol. 8, pp. 746-750.
Zhong, Q., et al., Science, 1999, vol. 285, pp. 747-750.
Tutt, A. and Ashworth, A., "The relationship between the roles of BRCA genes in DNA repair and cancer predisposition," TRENDS in Molecular Medicine, (2002) 8(12):571-576.
Dusemund, "Isochino [3,2-a]phthalazin-5,8-dione", *Arch. Pharm.*, (Weinhein) 1982, pp. 925-930. (English Abstract).
Ame , Bioessays (2004) 26(8):882-893.
Calabrese, Clin. Cancer Res. (2003) 9:2711-2718.
Caldecott, Cell (2003) 112:7-10.
Chalmers, Clin. Oncol. (2004) 16(1):29-39.
Chiarugi, Trends Pharmacol. Sci. (2002) 23:122-129.
D'Amours, Nat. Rev. Mol. Cell Biol. (2002) 3:317-327.
Dantzer, Biochimie (1999) 81:69-75.
Dusemund, Arch. Pharm. (1988) 321(1):41-44.
Egawa, Int. J. Cancer (2001) 95(4):255-259.
Egawa, Oncology (2001) 61(4):293-298.
El-Tamaty, Chem. Abs. (1996) 125(23):300924j.
Ferraris, J. et al., Med. Chem. (2003) 46:3138-3151.
Griffin, et al., Nat. Cell Biol. (2000) 5:757-761.
Grube, et al., Anal. Biochem. (1991) 193:236-239.
Halldorsson, et al., FEBS Lett. (1978) 85:349-352.
Herceg, Mutat. Res. (2001) 477:97-110.
Jackson, Carcinogenesis (2002) 23:687-696.
Jijon, et al., Am. J. Phsiol. Gastrointest. Liver Physiol. (2000) 279:G641-G651.
Johnson, et al., Nature (1999) 401:397-399.
Kanaar, et al., Trends Cell Biol. (1998) 8:483-489.
Kuperstein et al., Clin. Genet. (2000) 57(3):213-220.
Kupper et al., Cancer Res. (1996) 56:2715-2717.
Lindahl, Science (1999) 286:1897-1905.
Lindahl, Trends Biochem. Sci. (1995) 20:405-411.
Lundin et al., Mol. Cell Biol. (2002) 22:5869-5878.
Lundin et al., J. Mol. Biol. (2003) 328:521-535.
Magnussonet al., Mutagenesis (1990) 5:511-514.
McNealy et al., Anticancer Res. (2003) 23:1473-1478.
Menissier De Murcia, EMBO J. (2003) 22(9):2255-2263.
Morrison et al., Nature Gen. (1997) 17:479-482.
Nakamura et al., Nucleic Acids Res. (2003) 31:e104.
Pierce et al., Genes Dev. (1999) 13:2633-2638.
Samper et al., J. Cell. Biol. (2001) 154:49-60.
Satoh et al., Nature (1992) 356:356-358.
Schreiber, PNAS USA (1995) 92:4753-4757.
Schreiber et al., J. Biol. Chem. (2002) 277:23028-23036.
Seminov et al., Nucleic Acids Res. (1999) 27:4526-4531.
Shah et al., Biochim. Biophys. Acta. Mol. Cell Res. (1996) 1312:1-7.
Simbulan-Rosenthal, PNAS USA (1999) 96:13191-13196.
Szabo, "PARP as a Therapeutic Target," Zhang, Ed. CRC Press (2002) 169-204.
Tebbs, PNAS USA (1995) 92:6354-6358.
Tentori, Pharmacol. Res. (2002) 45:73-85.
Van Gent, Nat. Rev. Genet. (2001) 2:196-206.
Waldman, Nucleic Acids Res. (1991) 19:5943-5947.
Zhang, Portland Press Proc. (1999) 15:125.
Zingarelli, Immunology (2004) 113(4):509-517.

* cited by examiner

Primary Examiner—James O. Wilson
Assistant Examiner—Paul V. Ward
(74) *Attorney, Agent, or Firm*—Finnegan, Henderson, Farabow, Garrett & Dunner, LLP

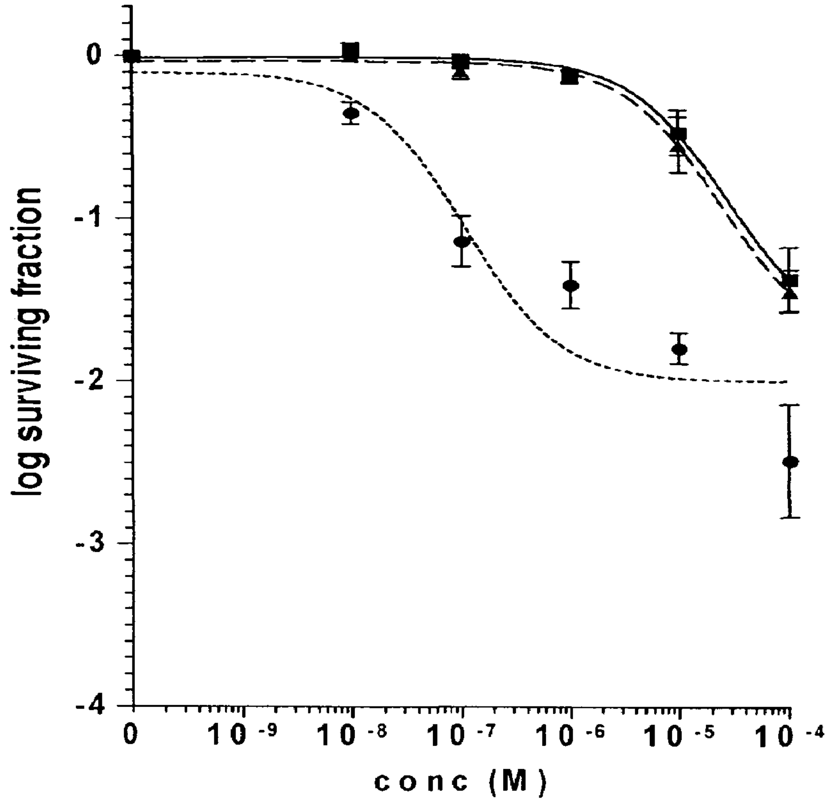
(57) **ABSTRACT**

Compounds of the formula (I):



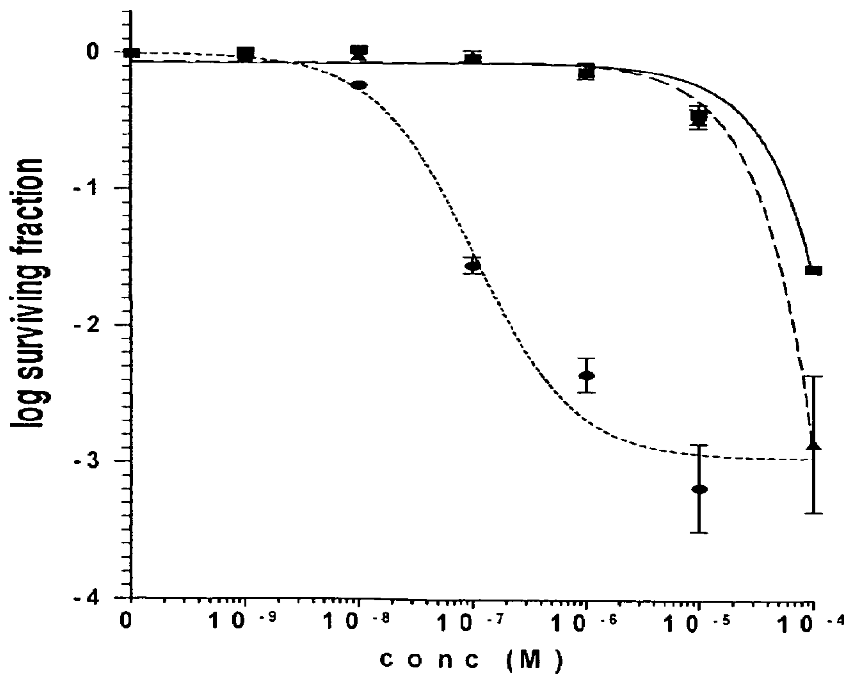
wherein A and B together represent an optionally substituted, fused aromatic ring; X can be NR^X or CR^XR^Y; if $X=NR^X$ then n is 1 or 2 and if $X=CR^XR^Y$ then n is 1; R^X is selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, amido, thioamido, ester, acyl, and sulfonyl groups; R^Y is selected from H, hydroxy, amino; or R^X and R^Y may together form a spiro-C_{3-7} cycloalkyl or heterocyclyl group; R^{C1} and R^{C2} are both hydrogen, or when X is CR^XR^Y, R^{C1}, R^{C2}, R^X and R^Y, together with the carbon atoms to which they are attached, may form an optionally substituted fused aromatic ring; and R^1 is selected from H and halo.

2 Claims, 2 Drawing Sheets



log surviving fraction
0
-1
-2
-3
-4
0
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
conc (M)

Fig. 1A



log surviving fraction
0
-1
-2
-3
-4
0
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
conc (M)

Fig. 1B

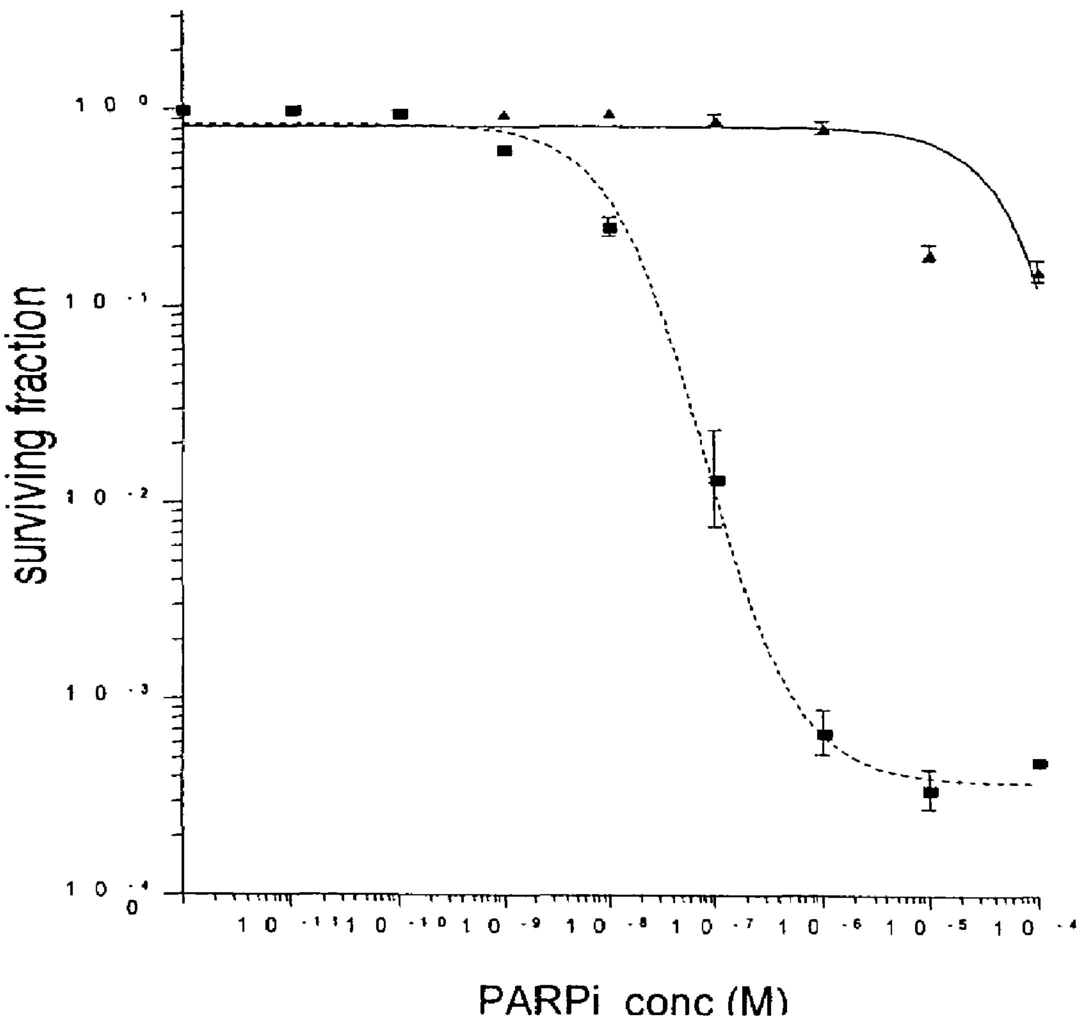

surviving fraction
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
0
10⁻¹¹
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
PARPi conc (M)

Fig. 2

PHTHALAZINONE DERIVATIVES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 10/799,154 filed Mar. 12, 2004, now abandoned which claimed the benefit of prior filed provisional patent application No. 60/454,995 filed on Mar. 14, 2003, and 60/526,244 filed on Dec. 1, 2003, and claimed the foreign priority of Great Britain Patent Application No. 0305681.9 filed on Mar. 12, 2003. This application also claims the benefit of prior filed provisional patent application No. 60/493,399 filed on August 6, 2003.

The present invention relates to phthalazinone derivatives, and their use as pharmaceuticals. In particular, the present invention relates to the use of these compounds to inhibit the activity of the enzyme poly(ADP-ribose)polymerase, also known as poly(ADP-ribose)synthase and poly ADP-ribosyltransferase, and commonly referred to as PARP.

The mammalian enzyme PARP (a 113-kDa multidomain protein) has been implicated in the signalling of DNA damage through its ability to recognize and rapidly bind to DNA single or double strand breaks (D'Amours, et al., *Biochem. J.,* 342, 249-268 (1999)).

Several observations have led to the conclusion that PARP participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also effects on telomere length and chromosome stability (d'Adda di Fagagna, et al., *Nature Gen.,* 23(1), 76-80 (1999)).

Studies on the mechanism by which PARP modulates DNA repair and other processes has identified its importance in the formation of poly(ADP-ribose) chains within the cellular nucleus (Althaus, F. R. and Richter, C., ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin (1987)). The DNA-bound, activated PARP utilizes NAD to synthesize poly (ADP-ribose) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Rhun, et al., *Biochem. Biophys. Res. Commun.,* 245, 1-10 (1998))

Poly(ADP-ribosyl)ation has also been associated with malignant transformation. For example, PARP activity is higher in the isolated nuclei of SV40-transformed fibroblasts, while both leukemic cells and colon cancer cells show higher enzyme activity than the equivalent normal leukocytes and colon mucosa (Miwa, et al., *Arch. Biochem. Biophys.,* 181, 313-321 (1977); Burzio, et al., *Proc. Soc. Exp. Bioi. Med.,* 149, 933-938 (1975); and Hirai, et al., *Cancer Res.,* 43, 3441-3446 (1983)).

A number of low-molecular-weight inhibitors of PARP have been used to elucidate the functional role of poly(ADP-ribosyl)ation in DNA repair. In cells treated with alkylating agents, the inhibition of PARP leads to a marked increase in DNA-strand breakage and cell killing (Durkacz, et al., *Nature,* 283, 593-596 (1980); Berger, N. A., *Radiation Research,* 101, 4-14 (1985)).

Subsequently, such inhibitors have been shown to enhance the effects of radiation response by suppressing the repair of potentially lethal damage (Ben-Hur, et al., *British Journal of Cancer,* 49 (Suppl. VI), 34-42 (1984); Schlicker, et al., *Int. J. Radiat. Bioi.,* 75, 91-100 (1999)). PARP inhibitors have been reported to be effective in radio sensitising hypoxic tumour cells (U.S. Pat. No. 5,032,617; U.S. Pat. No. 5,215,738 and U.S. Pat. No. 5,041,653).

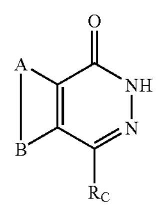
Furthermore, PARP knockout (PARP −/−) animals exhibit genomic instability in response to alkylating agents and γ-irradiation (Wang, et al., *Genes Dev.,* 9, 509-520 (1995); Menissier de Murcia, et al., *Proc. Natl. Acad. Sci. USA,* 94, 7303-7307 (1997)).

A role for PARP has also been demonstrated in certain vascular diseases, septic shock, ischaemic injury and neurotoxicity (Cantoni, et al., *Biochim. Biophys. Acta,* 1014, 1-7 (1989); Szabo, et al., *J. Clin. Invest.,* 100, 723-735 (1997)). Oxygen radical DNA damage that leads to strand breaks in DNA, which are subsequently recognised by PARP, is a major contributing factor to such disease states as shown by PARP inhibitor studies (Cosi, et al., *J. Neurosci. Res.,* 39, 38-46 (1994); Said, et al., *Proc. Natl. Acad. Sci. U.S.A.,* 93, 4688-4692 (1996)). More recently, PARP has been demonstrated to play a role in the pathogenesis of haemorrhagic shock (Liaudet, et al., *Proc. Natl. Acad. Sci. U.S.A.,* 97(3), 10203-10208 (2000)).

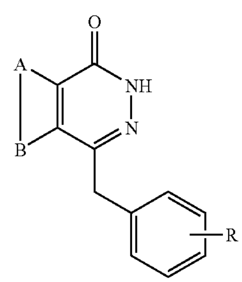
It has also been demonstrated that efficient retroviral infection of mammalian cells is blocked by the inhibition of PARP activity. Such inhibition of recombinant retroviral vector infections was shown to occur in various different cell types (Gaken, et al., *J. Virology,* 70(6), 3992-4000 (1996)). Inhibitors of PARP have thus been developed for the use in antiviral therapies and in cancer treatment (WO 91/18591).

Moreover, PARP inhibition has been speculated to delay the onset of aging characteristics in human fibroblasts (Rattan and Clark, *Biochem. Biophys. Res. Comm.,* 201(2), 665-672 (1994)). This may be related to the role that PARP plays in controlling telomere function (d'Adda di Fagagna, et al., *Nature Gen.,* 23(1), 76-80 (1999)).

Some of the present inventors have previously described (WO 02/36576) a class of 1(2H)-phthalazinone compounds which act as PARP inhibitors. The compounds have the general formula:



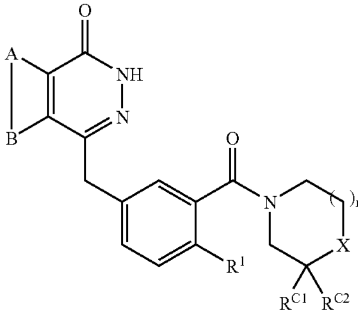
where A and B together represent an optional ly substitut ed, fused aromatic ring and where R_C is represented by -L-R_L. A large number of examples are of the formula:



where R represent one or more optional substituents.

The present inventors have now discovered that compounds where R is of a certain nature exhibit surprising levels of inhibition of the activity of PARP, and/or of potentiation of tumour cells to radiotherapy and various chemotherapies.

Accordingly, the first aspect of the present invention provides a compound of the formula (I):



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof

wherein:

A and B together represent an optionally substituted, fused aromatic ring;

X can be NR^X or CR^XR^Y;

if $X=NR^X$ then n is 1 or 2 and if $X=CR^XR^Y$ then n is 1;

R^X is selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, amido, thioamido, ester, acyl, and sulfonyl groups;

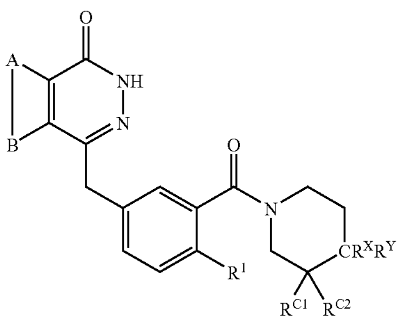
R^Y is selected from H, hydroxy, amino;

or R^X and R^Y may together form a spiro-C_{3-7} cycloalkyl or heterocyclyl group;

R^{C1} and R^{C2} are independently selected from the group consisting of hydrogen and C_{1-4} alkyl, or when X is CR^XR^Y, R^{C1}, R^{C2}, R^X and R^Y, together with the carbon atoms to which they are attached, may form an optionally substituted fused aromatic ring; and R^1 is selected from H and halo.

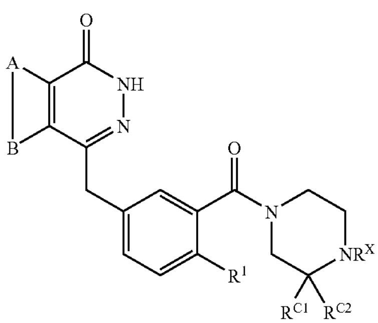
Therefore, if X is CR^XR^Y, then n is 1 and the compound is of formula (Ia):

(Ia)



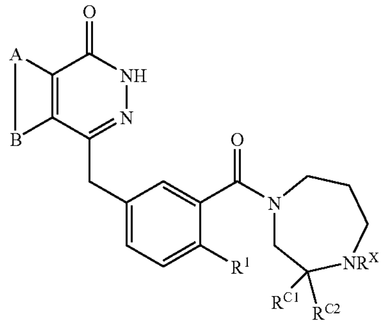
If X is NR^X, and n is 1, the compound is of formula (Ib):

(Ib)



If X is NR^X, and n is 2, the compound is of formula (Ic),.

(Ic)



A second aspect of the present invention provides a pharmaceutical composition comprising a compound of the first aspect and a pharmaceutically acceptable carrier or diluent.

A third aspect of the present invention provides the use of a compound of the first aspect in a method of treatment of the human or animal body.

A fourth aspect of the present invention provides the use of a compound as defined in the first aspect of the invention in the preparation of a medicament for:

(a) inhibiting the activity of PARP (PARP-1 and/or PARP-2);

(b) the treatment of: vascular disease; septic shock; ischaemic injury, both cerebral and cardiovascular; reperfusion injury, both cerebral and cardiovascular; neurotoxicity, including acute and chronic treatments for stroke and Parkinsons disease; haemorraghic shock; inflammatory diseases, such as arthritis; multiple sclerosis; secondary effects of diabetes; as well as the acute treatment of cytoxicity following cardiovascular surgery or diseases ameliorated by the inhibition of the activity of PARP;

(c) use as an adjunct in cancer therapy or for potentiating tumour cells for treatment with ionizing radiation or chemotherapeutic agents.

In particular, compounds as defined in the first aspect of the invention can be used in anti-cancer combination therapies (or as adjuncts) along with alkylating agents, such as methyl methanesulfonate (MMS), temozolomide and dacarbazine (DTIC), also with topoisomerase-1 inhibitors like Irinotecan, Rubitecan, Exatecan, Lurtotecan, Gimetecan, Diflomotecan (homocamptothecins); as well as 7-substituted non-silatecans; the 7-silyl camptothecins, BNP 1350; and non-camptothecin topoisomerase-I inhibitors such as indolocarbazoles also dual topoisomerase-I and II inhibitors like the benzophenazines, XR 11576/MLN 576 and benzopyridoindoles. Such combinations could be given, for example, as intravenous preparations or by oral administration as dependent on the preferred method of administration for the particular agent.

Other further aspects of the invention provide for the treatment of disease ameliorated by the inhibition of PARP, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect, preferably in the form of a pharmaceutical composition and the treatment of cancer, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect in combination, preferably in the form of a pharmaceutical composition, simultaneously or sequentially with ionizing radiation or chemotherapeutic agents.

In further aspects of the present invention, the compounds may be used in the preparation of a medicament for the

treatment of cancer which is deficient in Homologous Recombination (HR) dependent DNA double strand break (DSB) repair activity, or in the treatment of a patient with a cancer which is deficient in HR dependent DNA DSB repair activity, comprising administering to said patient a therapeutically-effective amount of the compound.

The HR dependent DNA DSB repair pathway repairs double-strand breaks (DSBs) in DNA via homologous mechanisms to reform a continuous DNA helix (K. K. Khanna and S. P. Jackson, Nat. Genet. 27(3): 247-254 (2001)). The components of the HR dependent DNA DSB repair pathway include, but are not limited to, ATM (NM_000051), RAD51 (NM_002875), RAD51L1 (NM_002877), RAD51C (NM_002876), RAD51L3 (NM_002878), DMC1 (NM_007068), XRCC2 (NM_005431), XRCC3 (NM 005432), RAD52 (NM_002879), RAD54L (NM_003579), RAD54B (NM_012415), BRCA1 (NM_007295), BRCA2 (NM_000059), RAD50 (NM_005732), MRE11A (NM_005590) and NBS1 (NM_002485). Other proteins involved in the HR dependent DNA DSB repair pathway include regulatory factors such as EMSY (Hughes-Davies, et al., *Cell,* 115, pp 523-535). HR components are also described in Wood, et al., *Science,* 291, 1284-1289 (2001).

A cancer which is deficient in HR dependent DNA DSB repair may comprise or consist of one or more cancer cells which have a reduced or abrogated ability to repair DNA DSBs through that pathway, relative to normal cells i.e. the activity of the HR dependent DNA DSB repair pathway may be reduced or abolished in the one or more cancer cells.

The activity of one or more components of the HR dependent DNA DSB repair pathway may be abolished in the one or more cancer cells of an individual having a cancer which is deficient in HR dependent DNA DSB repair. Components of the HR dependent DNA DSB repair pathway are well characterised in the art (see for example, Wood, et al., *Science,* 291, 1284-1289 (2001)) and include the components listed above.

In some preferred embodiments, the cancer cells may have a BRCA1 and/or a BRCA2 deficient phenotype i.e. BRCA1 and/or BRCA2 activity is reduced or abolished in the cancer cells. Cancer cells with this phenotype may be deficient in BRCA1 and/or BRCA2, i.e. expression and/or activity of BRCA1 and/or BRCA2 may be reduced or abolished in the cancer cells, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of amplification, mutation or polymorphism in a gene encoding a regulatory factor, for example the EMSY gene which encodes a BRCA2 regulatory factor (Hughes-Davies, et al., *Cell,* 115, 523-535).

BRCA1 and BRCA2 are known tumour suppressors whose wild-type alleles are frequently lost in tumours of heterozygous carriers (Jasin M., *Oncogene,* 21(58), 8981-93 (2002); Tutt, et al., *Trends Mol Med.,* 8(12), 571-6, (2002)). The association of BRCA1 and/or BRCA2 mutations with breast cancer is well-characterised in the art (Radice, P. J., *Exp Clin Cancer Res.,* 21(3 Suppl), 9-12 (2002)). Amplification of the EMSY gene, which encodes a BRCA2 binding factor, is also known to be associated with breast and ovarian cancer.

Carriers of mutations in BRCA1 and/or BRCA2 are also at elevated risk of cancer of the ovary, prostate and pancreas.

In some preferred embodiments, the individual is heterozygous for one or more variations, such as mutations and polymorphisms, in BRCA1 and/or BRCA2 or a regulator thereof. The detection of variation in BRCA1 and BRCA2 is well-known in the art and is described, for example in EP 699 754, EP 705 903, Neuhausen, S. L. and Ostrander, E. A., *Genet. Test,* 1, 75-83 (1992); Chappnis, P. O. and Foulkes, W. D., *Cancer Treat Res,* 107, 29-59 (2002); Janatova M., et al., *Neoplasma,* 50(4), 246-50 (2003); Jancarkova, N., *Ceska Gynekol.,* 68(1), 11-6 (2003)). Determination of amplification of the BRCA2 binding factor EMSY is described in Hughes-Davies, et al., *Cell,* 115, 523-535).

Mutations and polymorphisms associated with cancer may be detected at the nucleic acid level by detecting the presence of a variant nucleic acid sequence or at the protein level by detecting the presence of a variant (i.e. a mutant or allelic variant) polypeptide.

Definitions

The term "aromatic ring" is used herein in the conventional sense to refer to a cyclic aromatic structure, that is, a cyclic structure having delocalised π-electron orbitals.

The aromatic ring fused to the main core, i.e. that formed by -A-B-, may bear further fused aromatic rings (resulting in, e.g. naphthyl or anthracenyl groups). The aromatic ring(s) may comprise solely carbon atoms, or may comprise carbon atoms and one or more heteroatoms, including but not limited to, nitrogen, oxygen, and sulfur atoms. The aromatic ring(s) preferably have five or six ring atoms.

The aromatic ring(s) may optionally be substituted. If a substituent itself comprises an aryl group, this aryl group is not considered to be a part of the aryl group to which it is attached. For example, the group biphenyl is considered herein to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a phenyl group. Similarly, the group benzylphenyl is considered to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a benzyl group.

In one group of preferred embodiments, the aromatic group comprises a single aromatic ring, which has five or six ring atoms, which ring atoms are selected from carbon, nitrogen, oxygen, and sulfur, and which ring is optionally substituted. Examples of these groups include, but are not limited to, benzene, pyrazine, pyrrole, thiazole, isoxazole, and oxazole. 2-Pyrone can also be considered to be an aromatic ring, but is less preferred.

If the aromatic ring has six atoms, then preferably at least four, or even five or all, of the ring atoms are carbon. The other ring atoms are selected from nitrogen, oxygen and sulphur, with nitrogen and oxygen being preferred. Suitable groups include a ring with: no hetero atoms (benzene); one nitrogen ring atom (pyridine); two nitrogen ring atoms (pyrazine, pyrimidine and pyridazine); one oxygen ring atom (pyrone); and one oxygen and one nitrogen ring atom (oxazine).

If the aromatic ring has five ring atoms, then preferably at least three of the ring atoms are carbon. The remaining ring atoms are selected from nitrogen, oxygen and sulphur. Suitable rings include a ring with: one nitrogen ring atom (pyrrole); two nitrogen ring atoms (imidazole, pyrazole); one oxygen ring atom (furan); one sulphur ring atom (thiophene); one nitrogen and one sulphur ring atom (isothiazole, thiazole); and one nitrogen and one oxygen ring atom (isoxazole or oxazole).

The aromatic ring may bear one or more substituent groups at any available ring position. These substituents are selected from halo, nitro, hydroxy, ether, thiol, thioether, amino, C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl. The aromatic ring may also bear one or more substituent groups which together form a ring. In particular these may be of formula $—(CH_2)_m—$ or $—O—(CH_2)_p—O—$, where m is 2, 3, 4 or 5 and p is 1, 2 or 3.

Alkyl: The term "alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom

from a carbon atom of a hydrocarbon compound having from 1 to 20 carbon atoms (unless otherwise specified), which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term "alkyl" includes the sub-classes alkenyl, alkenyl, cycloalkyl, cycloalkyenyl, cylcoalkenyl, etc., discussed below.

In the context of alkyl groups, the prefixes (e.g. C_{1-4}, C_{1-7}, C_{1-20}, C_{2-7}, C_{3-7}, etc.) denote the number of carbon atoms, or range of number of carbon atoms. For example, the term "C_{1-4} alkyl", as used herein, pertains to an alkyl group having from 1 to 4 carbon atoms. Examples of groups of alkyl groups include C_{1-4} alkyl ("lower alkyl"), C_{1-7} alkyl, and C_{1-20} alkyl. Note that the first prefix may vary according to other limitations; for example, for unsaturated alkyl groups, the first prefix must be at least 2; for cyclic alkyl groups, the first prefix must be at least 3; etc.

Examples of (unsubstituted) saturated alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), propyl (C_3), butyl (C_4), pentyl (C_5), hexyl (C_6), heptyl (C_7), octyl (C_8), nonyl (C_9), decyl (C_{10}), undecyl (C_{11}), dodecyl (C_{12}), tridecyl (C_{13}), tetradecyl (C_{14}), pentadecyl (C_{15}), and eicodecyl (C_{20}).

Examples of (unsubstituted) saturated linear alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), n-propyl (C_3), n-butyl (C_4), n-pentyl (amyl) (C_5), n-hexyl (C_6), and n-heptyl (C_7).

Examples of (unsubstituted) saturated branched alkyl groups include, but are not limited to, iso-propyl (C_3), iso-butyl (C_4), sec-butyl (C_4), tert-butyl (C_4), iso-pentyl (C_5), and neo-pentyl (C_5).

Alkenyl: The term "alkenyl", as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds. Examples of alkenyl groups include C_{2-4} alkenyl, C_{2-7} alkenyl, C_{2-20} alkenyl.

Examples of (unsubstituted) unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, $—CH{=}CH_2$), 1-propenyl ($—CH{=}CH—CH_3$), 2-propenyl (allyl, $—CH—CH{=}CH_2$), isopropenyl (1-methylvinyl, $—C(CH_3){=}CH_2$), butenyl (C_4), pentenyl (C_5), and hexenyl (C_6).

Alkynyl: The term "alkynyl", as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds. Examples of alkynyl groups include C_{2-4} alkynyl, C_{2-7} alkynyl, C_{2-20} alkynyl.

Examples of (unsubstituted) unsaturated Alkenyl groups include, but are not limited to, ethynyl (ethinyl, $—C{\equiv}CH$) and 2-propynyl (propargyl, $—CH_2—C{\equiv}CH$).

Cycloalkyl: The term "cycloalkyl", as used herein, pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a carbocyclic ring of a carbocyclic compound, which carbocyclic ring may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated), which moiety has from 3 to 20 carbon atoms (unless otherwise specified), including from 3 to 20 ring atoms. Thus, the term "cycloalkyl" includes the sub-classes cycloalkenyl and cycloAlkenyl. Preferably, each ring has from 3 to 7 ring atoms. Examples of groups of cycloalkyl groups include C_{3-20} cycloalkyl, C_{3-15} cycloalkyl, C_{3-10} cycloalkyl, C_{3-7} cycloalkyl.

Examples of cycloalkyl groups include, but are not limited to, those derived from:

saturated monocyclic hydrocarbon compounds: cyclopropane (C_3), cyclobutane (C_4), cyclopentane (C_5), cyclohexane (C_6), cycloheptane (C_7), methylcyclopropane (C_4), dimethylcyclopropane (C_5), methylcyclobutane (C_5), dimethylcyclobutane (C_6), methylcyclopentane (C_6), dimethylcyclopentane (C_7), methylcyclohexane (C_7), dimethylcyclohexane (C_8), menthane (C_{10});

unsaturated monocyclic hydrocarbon compounds: cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclopropene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7), methylcyclohexene (C_7), dimethylcyclohexene (C_8);

saturated polycyclic hydrocarbon compounds: thujane (C_{10}), carane (C_{10}), pinane (C_{10}), bornane (C_{10}), norcarane (C_7), norpinane (C_7), norbornane (C_7), adamantane (C_{10}), decalin (decahydronaphthalene) (C_{10});

unsaturated polycyclic hydrocarbon compounds: camphene (C_{10}), limonene (C_{10}), pinene (C_{10});

polycyclic hydrocarbon compounds having an aromatic ring: indene (Cg), indane (e.g., 2,3-dihydro-1H-indene) (C_9), tetraline (1,2,3,4-tetrahydronaphthalene) (C_{10}), acenaphthene (C_{12}), fluorene (C_{13}), phenalene (C_{13}), acephenanthrene (C_{15}), aceanthrene (C_{16}), cholanthrene (C_{20}).

Heterocyclyl: The term "heterocyclyl", as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms (unless otherwise specified), of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

In this context, the prefixes (e.g. C_{3-20}, C_{3-7}, C_{5-6}, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C_{5-6}heterocyclyl", as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms. Examples of groups of heterocyclyl groups include C_{3-20} heterocyclyl, C_{5-20} heterocyclyl, C_{3-15} heterocyclyl, C_{5-15} heterocyclyl, C_{3-12} heterocyclyl, C_{5-12} heterocyclyl, C_{3-10} heterocyclyl, C_{5-10} heterocyclyl, C_{3-7} heterocyclyl, C_{5-7} heterocyclyl, and C_{5-6} heterocyclyl.

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from: N_1: aziridine (C_3), azetidine (C_4), pyrrolidine (tetrahydropyrrole) (C_5), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C_5), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C_5), piperidine (C_6), dihydropyridine (C_6) tetrahydropyridine (C_6), azepine (C_7); O_1: oxirane (C_3), oxetane (C_4), oxolane (tetrahydrofuran) (C_5), oxole (dihydrofuran) (C_5), oxane (tetrahydropyran) (C_6) dihydropyran (C_6), pyran (C_6), oxepin (C_7); S_1: thiirane (C_3), thietane (C_4), thiolane (tetrahydrothiophene) (C_5), thiane (tetrahydrothiopyran) (C_6), thiepane (C_7);

O_2: dioxolane (C_5), dioxane (C_6), and dioxepane (C_7);

O_3: trioxane (C_6);

N_2: imidazolidine (C_5), pyrazolidine (diazolidine) (C_5), imidazoline (C_5), pyrazoline (dihydropyrazole) (C_5), piperazine (C_6);

N_1O_1: tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);

N_1S_1: thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);

N_2O_1: oxadiazine (C_6);

O_1S_1: oxathiole (C_5) and oxathiane (thioxane) (C_6); and,

$N_1O_1S_1$: oxathiazine (C_6).

Examples of substituted (non-aromatic) monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses (C_5), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranse, and pyra-

noses (C_6), such as allopyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

Spiro-C_{3-7} cycloalkyl or heterocyclyl: The term "spiro C_{3-7} cycloalkyl or heterocyclyl" as used herein, refers to a C_{3-7} cycloalkyl or C_{3-7} heterocyclyl ring joined to another ring by a single atom common to both rings.

C_{5-20} aryl: The term "C_{5-20} aryl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups" in which case the group may conveniently be referred to as a "C_{5-20} carboaryl" group.

Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e. C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), and pyrene (C_{16}).

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulfur, as in "heteroaryl groups". In this case, the group may conveniently be referred to as a "C_{5-20} heteroaryl" group, wherein "C_{5-20}" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C_{5-20} heteroaryl groups include, but are not limited to, C_5 heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole), pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, tetrazole and oxatriazole; and C_6 heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) and triazine.

The heteroaryl group may be bonded via a carbon or hetero ring atom.

Examples of C_{5-20} heteroaryl groups which comprise fused rings, include, but are not limited to, C_9 heteroaryl groups derived from benzofuran, isobenzofuran, benzothiophene, indole, isoindole; C_{10} heteroaryl groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine; C_{14} heteroaryl groups derived from acridine and xanthene.

The above alkyl, heterocyclyl, and aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: —F, —Cl, —Br, and —I.

Hydroxy: —OH.

Ether: —OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkoxy group), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7} alkyl group.

Nitro: —NO_2.

Cyano (nitrile, carbonitrile): —CN.

Acyl (keto): —C(═O)R, wherein R is an acyl substituent, for example, H, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, —C(═O)CH_3 (acetyl), —C(═O)CH_2CH_3 (propionyl), —C(═O)C$(CH_3)_3$ (butyryl), and —C(═O)Ph (benzoyl, phenone).

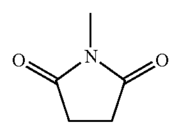
Carboxy (carboxylic acid): —COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): —C(═O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, —C(═O)OCH_3, —C(═O)OCH_2CH_3, —C(═O)OC$(CH_3)_3$, and —C(═O)OPh.

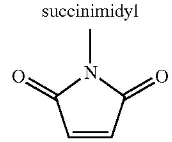
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): —C(═O)NR^1R^2, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, —C(═O)NH_2, —C(═O)$NHCH_3$, —C(═O)N$(CH_3)_2$, —C(═O)$NHCH_2CH_3$, and —C(═O)N$(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinylcarbonyl.

Amino: —NR^1R^2, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, —NH_2, —$NHCH_3$, —NHCH$(CH_3)_2$, —N$(CH_3)_2$, —N$(CH_2CH_3)_2$, and —NHPh. Examples of cyclic amino groups include, but are not limited to, aziridinyl, azetidinyl, pyrrolidinyl, piperidino, piperazinyl, perhydrodiazepinyl, morpholino, and thiomorpholino. In particular, the cyclic amino groups may be substituted on their ring by any of the substituents defined here, for example carboxy, carboxylate and amido.

Acylamido (acylamino): —NR^1C(═O)R^2, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, most preferably H, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acylamide groups include, but are not limited to, —NHC(═O)CH_3, —NHC(═O)CH_2CH_3, and —NHC(═O)Ph. R^1 and R^2 may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl, and phthalimidyl:

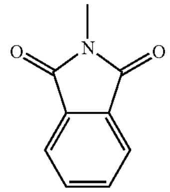


succinimidyl



maleimidyl

-continued



phthalimidyl

Ureido: —N(R^1)CONR^{2}R^3 wherein R^2 and R^3 are independently amino substituents, as defined for amino groups, and R1 is a ureido substituent, for example, hydrogen, a C_{1-7}alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20}aryl group, preferably hydrogen or a C_{1-7}alkyl group. Examples of ureido groups include, but are not limited to, —NHCONH$_2$, —NHCONHMe, —NHCONHEt, —NHCONMe$_2$, —NHCONEt$_2$, —NMeCONH$_2$, —NMeCONHMe, —NMeCONHEt, —NMeCONMe$_2$, —NMeCONEt$_2$ and —NHCONHPh.

Acyloxy (reverse ester): —OC(═O)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acyloxy groups include, but are not limited to, —OC(═O)CH$_3$ (acetoxy), —OC(═O)CH$_2$CH$_3$, —OC(═O)C(CH$_3$)$_3$, —OC(═O)Ph, —OC(═O)C$_6$H$_4$F, and —OC(═O)CH$_2$Ph.

Thiol: —SH.

Thioether (sulfide): —SR, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, —SCH$_3$ and —SCH$_2$CH$_3$.

Sulfoxide (sulfinyl): —S(═O)R, wherein R is a sulfoxide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfoxide groups include, but are not limited to, —S(═O)CH$_3$ and —S(═O)CH$_2$CH$_3$.

Sulfonyl (sulfone): —S(═O)$_2$R, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, —S(═O)$_2$CH$_3$ (methanesulfonyl, mesyl), —S(═O)$_2$CF$_3$, —S(═O)$_2$CH$_2$CH$_3$, and 4-methylphenylsulfonyl (tosyl).

Thioamido (thiocarbamyl): —C(═S)NR^{1}R^2, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, —C(═S)NH$_2$, —C(═S)NHCH$_3$, —C(═S)N(CH$_3$) 2, and —C(═S)NHCH$_2$CH$_3$.

Sulfonamino: —NR^{1}S(═O)$_2$R, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7}alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20}aryl group, preferably a C_{1-7}alkyl group. Examples of sulfonamino groups include, but are not limited to, —NHS(═O)$_2$CH$_3$, —NHS(═O)$_2$Ph and —N(CH$_3$)S(═O)$_2$C$_6$H$_5$.

As mentioned above, the groups that form the above listed substituent groups, e.g. C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl, may themselves be substituted. Thus, the above definitions cover substituent groups which are substituted.

BRIEF DESCRIPTION OF FIGURES

FIG. **1** shows clonogenic survival curves of cells proficient and deficient in Braca 1 exposed to compound (4) of the present invention.

FIG. **1**A shows Brca1 wild type (11CO:■), heterozygous (Cre6:▲) and deficient (Cre10:●) embryonic fibroblastic stem (ES) cells under continuous exposure to compound 4. Error bars represent standard errors of the mean.

FIG. **1**B shows Brca2 wild type (D3:■), heterozygous (Cre6:▲) and deficient (Cre24:●) ES cells under continuous exposure to compound 4. Error bars represent standard errors of the mean.

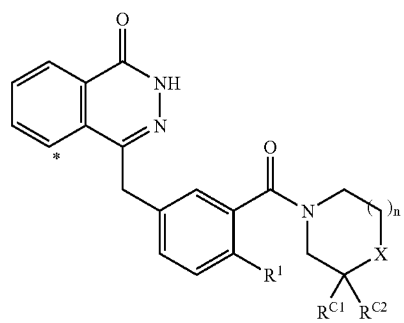
FIG. **2** shows an analysis of the effects of a compound of the invention (4) in another cell line lacking BRCA2 function in comparison to a BRCA2 complemented line. Data shown is clonogenic survival of Brca2 deficient (V-C8:■) and complemented (V-C8 BAC+:▲) cells under continuous exposure to compound 4 at varying concentrations.

FURTHER PREFERENCES

The following preferences can apply to each aspect of the present invention, where applicable.

In the present invention, the fused aromatic ring(s) represented by -A-B- preferably consist of solely carbon ring atoms, and thus may be benzene, naphthalene, and is more preferably benzene. As described above, these rings may be substituted, but in some embodiments are preferably unsubstituted.

If the fused aromatic ring represented by -A-B- bears a substituent group, it is preferably attached to the atom which itself is attached to the central ring meta- to the carbonyl group. Thus, if the fused aromatic ring is a benzene ring, the preferred place of substitution is shown in the formula below by *:



which is usually termed the 5-position of the phthalazinone moiety.

R^1 is preferably selected from H, Cl and F, and is more preferably F.

It is preferred that R^{C1} and R^{C2} are independently selected from hydrogen and C_{1-4} alkyl, and more preferably H and methyl. It is more preferred that at least one of R^{C1} and R^{C2} are hydrogen, with the most preferred option being that both are hydrogen.

When n is 2, X is NR^X. In these embodiments, R^X is preferably selected from the group consisting of: H; optionally substituted C_{1-20} alkyl (for example, optionally substituted C_{5-20} arylmethyl); optionally substituted C_{5-20} aryl; optionally substituted ester groups, wherein the ester substituent is preferably C_{1-20} alkyl; optionally substituted acyl groups; option-

ally substituted amido groups; optionally substituted thioamido groups; and optionally substituted sulfonyl groups. R^X is more preferably selected from the group consisting of: H; optionally substituted C_{1-20} alkyl; optionally substituted C_{5-20} aryl; and optionally substituted ester groups, wherein the ester substituent is preferably C_{1-20} alkyl.

When n is 1, X may be NR^X or CR^XCR^Y.

In embodiments where X is NR^X, R^X is preferably selected from the group consisting of: H; optionally substituted C_{1-20} alkyl (for example, optionally substituted C_{5-20} arylmethyl); optionally substituted C_{5-20} aryl; optionally substituted acyl; optionally substituted sulfonyl; optionally substituted amido; and optionally substituted thioamido groups.

In embodiments where X is CR^XR^Y, R^Y is preferably H. R^X is preferably selected from the group consisting of: H; optionally substituted C_{1-20} alkyl (for example, optionally substituted C_{5-20} arylmethyl); optionally substituted C_{5-20} aryl; optionally substituted C_{3-20} heterocyclyl; optionally substituted acyl, wherein the acyl substituent is preferably selected from C_{5-20} aryl and C_{3-20} heterocylyl (e.g. piperazinyl); optionally substituted amido, wherein the amino groups are preferably selected from H and C_{1-20} alkyl or together with the nitrogen atom, form a C_{5-20} heterocyclic group; and optionally substituted ester groups, wherein the ester substituent is preferably selected from C_{1-20} alkyl groups.

Particularly preferred compounds include: 1, 2, 3, 4, 10, 20, 59, 80, 135, 146, 192, 194, 195, 211 and 212.

Where appropriate, the above preferences may be taken in combination with each other.

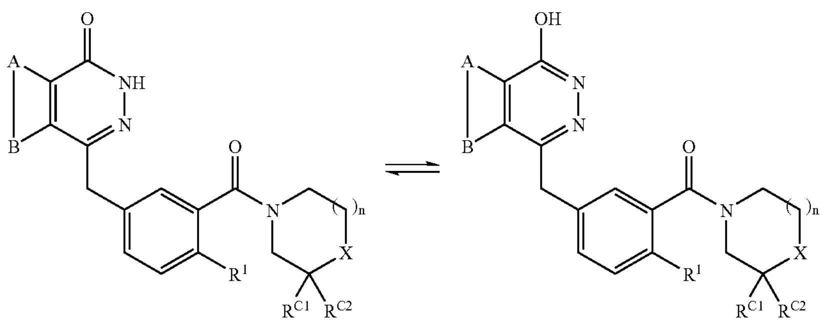
forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (−) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

If the compound is in crystalline form, it may exist in a number of different polymorphic forms.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, —OCH_3, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, —CH_2OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol, imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

Particularly relevant to the present invention is the tautomeric pair illustrated below:



Includes Other Forms

Included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (—COOH) also includes the anionic (carboxylate) form (—COO^-), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form (—$N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form (—O^-), a salt or solvate thereof, as well as conventional protected forms of a hydroxyl group.

Isomers, Salts, Solvates, Protected Forms, and Prodrugs

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasterioisomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r-forms; endo- and exo-

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^1H, ^2H (D), and ^3H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below, as well as its different polymorphic forms.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, et al., "Pharmaceutically Acceptable Salts", *J. Pharm. Sci.*, 66, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., —COOH may be —COO$^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g., —NH_2 may be —NH_3^+), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, gycolic, stearic, palmitic, lactic, malic, pamoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicyclic, sulfanilic, 2-acetyoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, isethionic, valeric, and gluconic. Examples of suitable polymeric anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form," as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, "Protective Groups in Organic Synthesis" (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

For example, a hydroxy group may be protected as an ether (—OR) or an ester (—OC(═O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (—OC(═O)CH_3, —OAc).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group (>C═O) is converted to a diether (>C$(OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide (—NHCO—CH_3); a benzyloxy amide (—NHCO—$OCH_2C_6H_5$, —NH-Cbz); as a t-butoxy amide (—NHCO—$OC(CH_3)_3$, —NH-Boc); a 2-biphenyl-2-propoxy amide (—NHCO—$OC(CH_3)_2C_6H_4C_6H_5$, —NH-Bpoc), as a 9-fluorenylmethoxy amide (—NH-Fmoc), as a 6-nitroveratryloxy amide (—NH-Nvoc), as a 2-trimethylsilylethyloxy amide (—NH-Teoc), as a 2,2,2-trichloroethyloxy amide (—NH-Troc), as an allyloxy amide (—NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (—NH-Psec); or, in suitable cases, as an N-oxide (>NO□).

For example, a carboxylic acid group may be protected as an ester for example, as: a C_{1-7} alkyl ester (e.g. a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g. a C_{1-7} trihaloalkyl ester); a triC_{1-7} alkylsilyl-C_{1-7} alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (—SR), for example, as: a benzyl thioether; an acetamidomethyl ether (—S—CH_2NHC(═O)CH_3)

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug", as used herein, pertains to a compound which, when metabolised (e.g. in vivo), yields the desired active compound. Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

For example, some prodrugs are esters of the active compound (e.g. a physiologically acceptable metabolically labile ester). During metabolism, the ester group (—C(═O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (—C(═O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required. Examples of such metabolically labile esters include, but are not limited to, those wherein R is C_{1-20} alkyl (e.g. -Me, -Et); C_{1-7} aminoalkyl (e.g. aminoethyl; 2-(N,N-diethylamino)ethyl; 2-(4-morpholino)ethyl); and acyloxy-C_{1-7} alkyl (e.g. acyloxymethyl; acyloxyethyl; e.g. pivaloyloxymethyl; acetoxymethyl; 1-acetoxyethyl; 1-(1-methoxy-1-methyl)ethyl-carbonxyloxyethyl; 1-(benzoyloxy)ethyl; isopropoxy-carbonyloxymethyl; 1-isopropoxy-carbonyloxyethyl; cyclohexyl-carbonyloxymethyl; 1-cyclohexyl-carbonyloxyethyl; cyclohexyloxy-carbonyloxymethyl; 1-cyclohexyloxy-carbonyloxyethyl; (4-tetrahydropyranyloxy) carbonyloxymethyl; 1-(4-tetrahydropyranyloxy) carbonyloxyethyl; (4-tetrahydropyranyl) carbonyloxymethyl; and 1-(4-tetrahydropyranyl) carbonyloxyethyl).

Further suitable prodrug forms include phosphonate and glycolate salts. In particular, hydroxy groups (—OH), can be made into phosphonate prodrugs by reaction with chlorodibenzylphosphite, followed by hydrogenation, to form a phosphonate group —O—P(═O)$(OH)_2$. Such a group can be cleaved by phosphatase enzymes during metabolism to yield the active drug with the hydroxy group.

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further

chemical reaction, yields the active compound. For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Acronyms

For convenience, many chemical moieties are represented using well known abbreviations, including but not limited to, methyl (Me), ethyl (Et), n-propyl (nPr), iso-propyl (iPr), n-butyl (nBu), tert-butyl (tBu), n-hexyl (nHex), cyclohexyl (cHex), phenyl (Ph), biphenyl (biPh), benzyl (Bn), naphthyl (naph), methoxy (MeO), ethoxy (EtO), benzoyl (Bz), and acetyl (Ac).

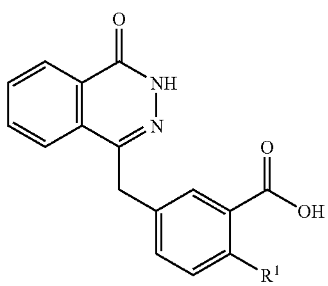
For convenience, many chemical compounds are represented using well known abbreviations, including but not limited to, methanol (MeOH), ethanol (EtOH), iso-propanol (i-PrOH), methyl ethyl ketone (MEK), ether or diethyl ether (Et_2O), acetic acid (AcOH), dichloromethane (methylene chloride, DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), tetrahydrofuran (THF), and dimethylsulfoxide (DMSO).

Synthesis

In the synthesis routes given below, the A-B fused ring is shown as a fused benzene ring for convenience. Compounds in which the A-B ring is other than benzene may be synthesised using methodologies analogous to those described below by the use of appropriate alternative starting materials.

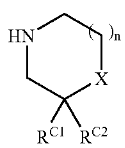
Compounds of the present invention may be synthesised by reaction of a compound of Formula 1:

Formula 1



in which R^1 is as previously defined, with a compound of Formula 2:

Formula 2

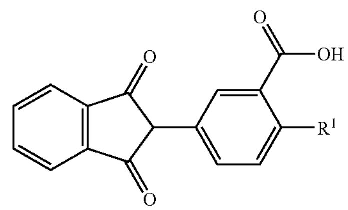


in which n, R^{C1}, R^{C2} and X are as previously defined, in the presence of a coupling reagent system, for example 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or (dimethylaminopropyl) ethylcarbodiimide hydrochloride/hydroxybenzotriazole, in the presence of a base, for example diisopropylethylamine, in a solvent, for example dimethylacetamide or dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Alternatively, compounds of the present invention may be synthesised by conversion of a compound of Formula 1 into an activated species, for example an acid chloride or an activated ester such as an N-hydroxysuccinimide ester, using well-known methodologies, and reaction of the activated species with a compound of Formula 2.

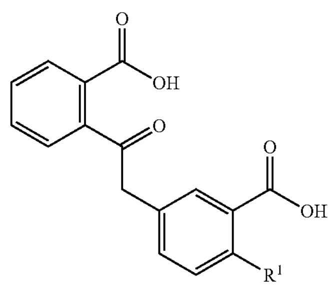
Compounds of Formula 1 may be synthesised by reaction of a compound of Formula 3:

Formula 3



in which R^1 is as previously defined, or a compound of Formula 4:

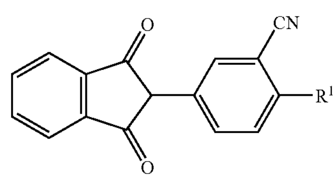
Formula 4



in which R^1 is as previously defined, or a mixture of a compound of Formula 3 and a compound of Formula 4, with a source of hydrazine, for example hydrazine hydrate, optionally in the presence of a base, for example triethylamine, optionally in the presence of a solvent, for example industrial methylated spirit, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula 3 or Formula 4, or mixtures thereof, may be synthesised by reaction of a compound of Formula 5:

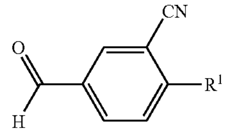
Formula 5



in which R^1 is as previously defined, with a reagent capable of hydrolysing a nitrile moiety, for example sodium hydroxide, in the presence of a solvent, for example water, at a temperature in the range of 0° C. to the boiling point of the solvent used.

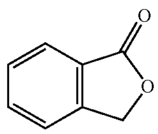
Compounds of Formula 5 may be synthesised by reaction of a compound of Formula 6:

Formula 6



in which R^1 is as previously defined, with a compound of Formula 7:

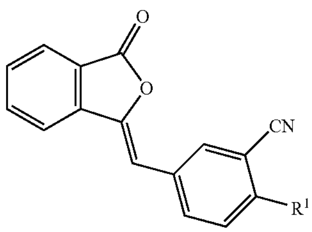
Formula 7



in the presence of a base, for example sodium methoxide, in a solvent, for example methanol, optionally in the presence of a water scavenger, for example ethyl propionate, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula 1 may also be synthesised by reaction of a compound of Formula 8:

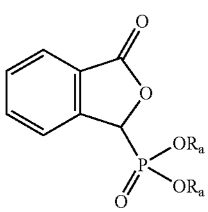
Formula 8



in which R^1 is as previously defined, with a reagent capable of hydrolysing a nitrile moiety, for example sodium hydroxide, in the presence of a solvent, for example water, at a temperature in the range of 0° C. to the boiling point of the solvent used, followed by reaction of the resulting intermediate with a source of hydrazine, for example hydrazine hydrate, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula 8 may be synthesised by reaction of a compound of Formula 9:

Formula 9



in which R_a is a C_{1-4} alkyl group, with a compound of Formula 6, in the presence of a base, for example triethylamine or lithium hexamethyldisilazide, in the presence of a solvent, for example tetrahydrofuran, at a temperature in the range of −80° C. to the boiling point of the solvent used.

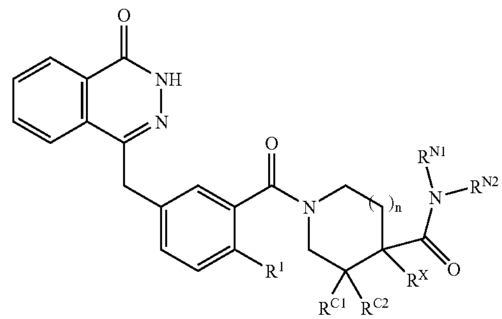
Compounds of Formula 9 may be synthesised by methods analogous to those described in WO 02/26576.

Compounds of Formula 1 may also be synthesised by methods analogous to those described above in which the nitrile moiety in all Formulae is replaced by other moieties capable of generating a carboxylic acid, for example ester or carboxamide moieties.

Compounds of Formula 2 are commercially available or may be synthesised by methods reported in the chemical literature.

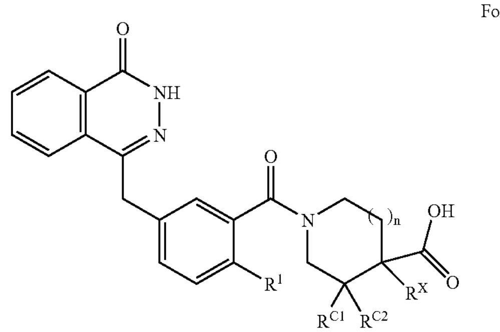
Compounds of the present invention in which X is CR^XR^Y, in which one of R^X or R^Y is an amido moiety, and which may therefore be represented by Formula 10:

Formula 10



in which n, R^{C1}, R^{C2}, R^1 and R^X are as previously defined and R^{N1} and R^{N2} are each individually selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, or may together form an optionally substituted C_{3-7} cycloalkyl or heterocyclyl group, may be synthesised by reaction of a compound of Formula 11:

Formula 11

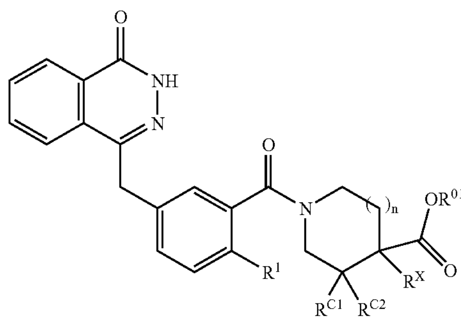


in which n, R^{C1}, R^{C2}, R^1 and R^X are as previously defined, with a compound of Formula $HNR^{N1}R^{N2}$, in which R^{N1} and R^{N2} are as previously defined, in the presence of a coupling reagent system, for example 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or (dimethylaminopropyl)ethylcarbodiimide hydrochloride/hydroxybenzotriazole, in the presence of a base, for example diisopropylethylamine, in a solvent, for example dimethylacetamide or dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Alternatively, compounds of Formula 10 may be synthesised by conversion of a compound of Formula 11 into an activated species, for example an acid chloride or an activated ester such as an N-hydroxysuccinimide ester, using well-known methodologies, and reaction of the activated species with a compound of Formula $HNR^{N1}R^{N2}$.

Compounds of Formula 11 may be synthesised by deprotection of a protected form of a compound of Formula 11, for example a compound of Formula 12:

Formula 12



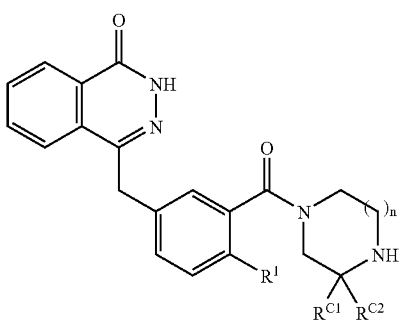
in which n, R^{C1}, R^{C2}, R^1 and R^X are as previously defined and R^{01} is a C_{1-4} alkyl group, using well known methodologies, for example base-catalysed hydrolysis in the presence of a source of hydroxide, for example sodium or lithium hydroxide, in the presence of a solvent, for example water and/or tetrahydrofuran, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula 12 may be synthesised from compounds of Formula 1 by the previously described methods.

Compounds of Formula $HNR^{N1}R^{N2}$ are commercially available or may be synthesised by methods reported in the chemical literature.

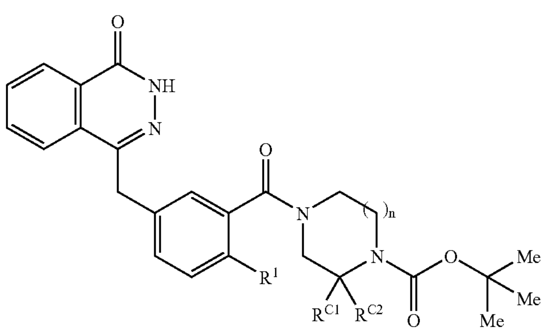
Compounds of the present invention in which X is NH and which may therefore be represented by Formula 13:

Formula 13



in which n, R^{C1}, R^{C2} and R^1 are as previously defined, may be synthesised by deprotection of a protected form of a compound of Formula 13, for example a compound of Formula 14:

Formula 14

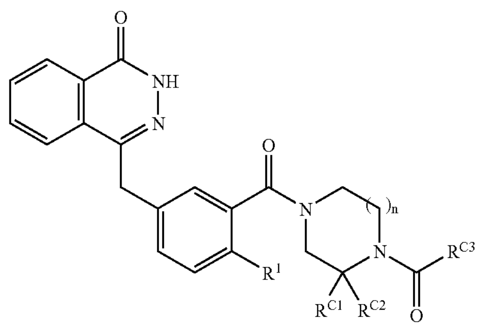


in which n, R^{C1}, R^{C2} and R^1 are as previously defined, using well known methodologies, for example acid-catalysed cleavage, in the presence of an acid, for example trifluoroacetic acid or hydrochloric acid, in the presence of a solvent, for example dichloromethane or ethanol and/or water, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula 14 may be synthesised from compounds of Formula 1 by the previously described methods.

Compounds of the present invention in which X is NR^X, in which R^X is an acyl moiety, and which may therefore be represented by Formula 15:

Formula 15



in which n, R^{C1}, R^{C2} and R^{01} are as previously defined and R^{C3} is selected from the group consisting of optionally substituted C_{1-20} alkyl, C_{5-20} aryl and C_{3-20} heterocyclyl, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula $R^{C3}COX$, in which R^{C3} is as previously defined and X is a suitable leaving group, for example a halogen such as chloro, optionally in the presence of a base, for example pyridine, triethylamine or diisopropylethylamine, optionally in the presence of a. solvent, for example dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

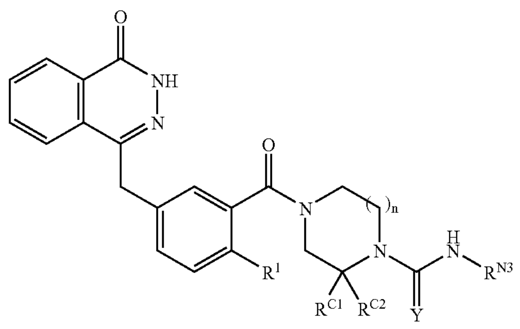
Compounds of Formula $R^{C3}COX$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of Formula 15 may also be synthesised by reaction of a compound of Formula 13 with a compound of Formula $R^{C3}CO_2H$, in which R^{C3} is as previously defined, in the presence of a coupling reagent system, for example 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or (dimethylaminopropyl)ethylcarbodiimide hydrochloride/hydroxybenzotriazole, in the presence of a base, for example diisopropylethylamine, in a solvent, for example dimethylacetamide or dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula $R^{C3}CO_2H$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^X, in which R^X is an amido or thioamido moiety, and which may therefore be represented by Formula 16:

Formula 16

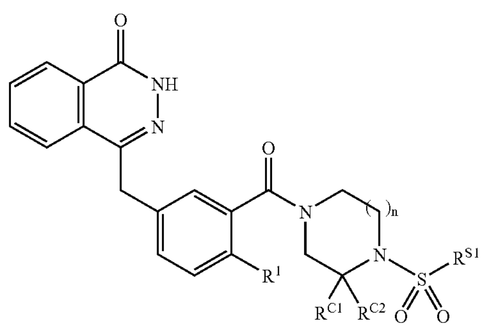


in which n, R^{C1}, R^{C2} and R^1 are as previously defined, Y is O or S and R^{N3} is selected from the group consisting of optionally substituted C_{1-20} alkyl, C_{5-20} aryl and C_{3-20} heterocyclyl, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula R^{N3} NCY, in which Y and R^{N3} are as previously defined, in the presence of a solvent, for example dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula R^{N3}NCY are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^X, in which R^X is a sulfonyl moiety, and which may therefore be represented by Formula 17:

Formula 17

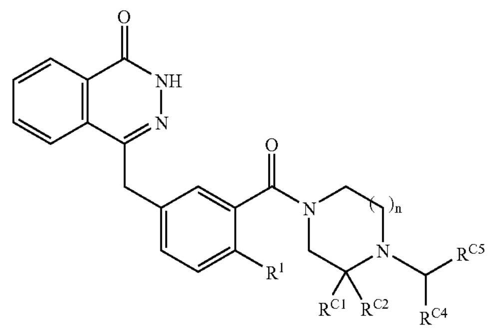


in which n, R^{C1}, R^{C2} and R^1 are as previously defined and R^{S1} is selected from the group consisting of optionally substituted C_{1-20} alkyl, C_{5-20} aryl and C_{3-20} heterocyclyl, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula $R^{S1}SO_2Cl$, in which R^{S1} is as previously defined, optionally in the presence of a base, for example pyridine, triethylamine or diisopropylethylamine, in the presence of a solvent, for example dichloromethane, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula $R^{S1}O_2Cl$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^X, in which R^X is selected from the group consisting of optionally substituted C_{1-20} alkyl or C_{3-20} heterocyclyl, and which may therefore be represented by Formula 18:

Formula 18



in which n, R^{C1}, R^{C2} and R^1 are as previously defined and R^{C4} and R^{C5} are each individually selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, or may together form an optionally substituted C_{3-7} cycloalkyl or heterocyclyl group, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula R^{C4} COR^{C5}, in which R^{C4} and R^{C5} are as previously defined, in the presence of a reducing agent, for example sodium cyanoborohydride or sodium triacetoxyborohydride, in the presence of a solvent, for example methanol, optionally in the presence of an acid catalyst, for example acetic acid, at a temperature in the range of 0° C. to the boiling point of the solvent used.

Compounds of Formula R^{C4} COR^{C5} are commercially available or may be synthesised by methods reported in the chemical literature.

Use

The present invention provides active compounds, specifically, active in inhibiting the activity of PARP.

The term “active” as used herein, pertains to compounds which are capable of inhibiting PARP activity, and specifically includes both compounds with intrinsic activity (drugs) as well as prodrugs of such compounds, which prodrugs may themselves exhibit little or no intrinsic activity.

One assay which may conveniently be used in order to assess the PARP inhibition offered by a particular compound is described in the examples below.

The present invention further provides a method of inhibiting the activity of PARP in a cell, comprising contacting said cell with an effective amount of an active compound, preferably in the form of a pharmaceutically acceptable composition. Such a method may be practised in vitro or in vivo.

For example, a sample of cells may be grown in vitro and an active compound brought into contact with said cells, and the effect of the compound on those cells observed. As examples of “effect”, the amount of DNA repair effected in a certain time may be determined. Where the active compound is found to exert an influence on the cells, this may be used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

The term “treatment”, as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and

cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

The term "adjunct" as used herein relates to the use of active compounds in conjunction with known therapeutic means. Such means include cytotoxic regimens of drugs and/or ionising radiation as used in the treatment of different cancer types. In particular, the active compounds are known to potentiate the actions of a number of cancer chemotherapy treatments, which include the topoisomerase class of poisons and most of the known alkylating agents used in treating cancer.

Active compounds may also be used as cell culture additives to inhibit PARP, for example, in order to sensitize cells to known chemotherapeutic agents or ionising radiation treatments in vitro.

Active compounds may also be used as part of an in vitro assay, for example, in order to determine whether a candidate host is likely to benefit from treatment with the compound in question.

Administration

The active compound or pharmaceutical composition comprising the active compound may be administered to a subject by any convenient route of administration, whether systemically/peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutang, gibbon), or a human.

Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, "Handbook of Pharmaceutical Additives", 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse Information Resources, Inc., Endicott, N.Y., USA), "Remington's Pharmaceutical Sciences", 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and "Handbook of Pharmaceutical Excipients", 2nd edition, 1994.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, losenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

A tablet may be made by conventional means, e.g. compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration (e.g. transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol, or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active compounds and optionally one or more excipients or diluents.

Formulations suitable for topical administration in the mouth include losenges comprising the active compound in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active compound in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active compound in a suitable liquid carrier.

Formulations suitable for topical administration to the eye also include eye drops wherein the active compound is dis-

solved or suspended in a suitable carrier, especially an aqueous solvent for the active compound.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebuliser, include aqueous or oily solutions of the active compound.

Formulations suitable for administration by inhalation include those presented as an aerosol spray from a pressurised pack, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichorotetrafluoroethane, carbon dioxide, or other suitable gases.

Formulations suitable for topical administration via the skin include ointments, creams, and emulsions. When formulated in an ointment, the active compound may optionally be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active compounds may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active compound through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogues.

When formulated as a topical emulsion, the oily phase may optionally comprise merely an emulsifier (otherwise known as an emulgent), or it may comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabiliser. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabiliser(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Suitable emulgents and emulsion stabilisers include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulphate. The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations may be very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active compound, such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration (e.g., by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 μg/ml, for example from about 10 ng/ml to about 1 μg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

Dosage

It will be appreciated that appropriate dosages of the active compounds, and compositions comprising the active compounds, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration in vivo can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

In general, a suitable dose of the active compound is in the range of about 100 μg to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered

is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

Synthesis Data

General Experimental Methods

Preparative HPLC

Samples were purified with a Waters mass-directed purification system utilising a Waters 600 LC pump, Waters Xterra C18 column (5 μm 19 mm×50 mm) and Micromass ZQ mass spectrometer, operating in positive ion electrospray ionisation mode. Mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) were used in a gradient; 5% B to 100% over 7 min, held for 3 min, at a flow rate of 20 ml/min.

Analytical HPLC-MS

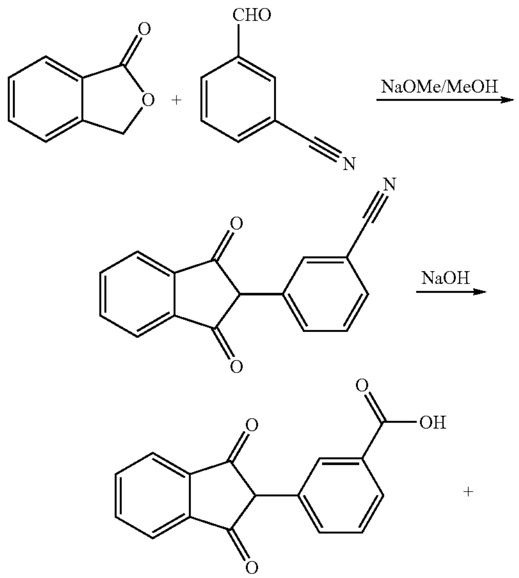
Analytical HPLC was typically carried out with a Spectra System P4000 pump and Jones Genesis C18 column (4 μm, 50 mm×4.6 mm). Mobile phases A (0.1% formic acid in water) and B (acetonitrile) were used in a gradient of 5% B for 1 min rising to 98% B after 5 min, held for 3 min at a flow rate of 2 ml/min. Detection was by a TSP UV 6000LP detector at 254 nm UV and range 210-600 nm PDA. The Mass spectrometer was a Finnigan LCQ operating in positive ion electrospray mode.

NMR

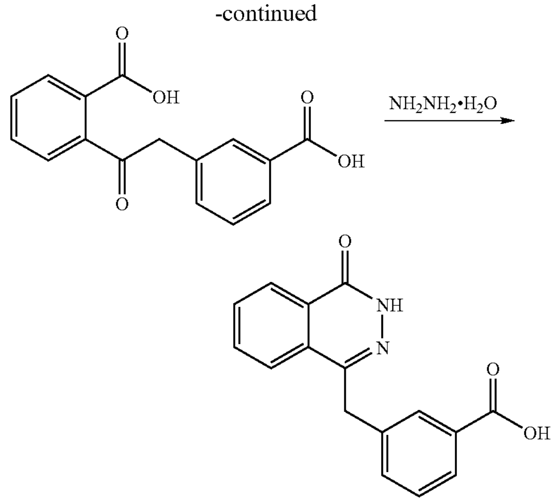
^{1}H NMR and ^{13}C NMR were typically recorded using Bruker DPX 300 spectrometer at 300 MHz and 75 MHz respectively. Chemical shifts were reported in parts per million (ppm) on the δ scale relative to tetramethylsilane internal standard. Unless stated otherwise all samples were dissolved in DMSO-d_6.

Synthesis of Key Intermediates

a. 3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)



-continued



(A)

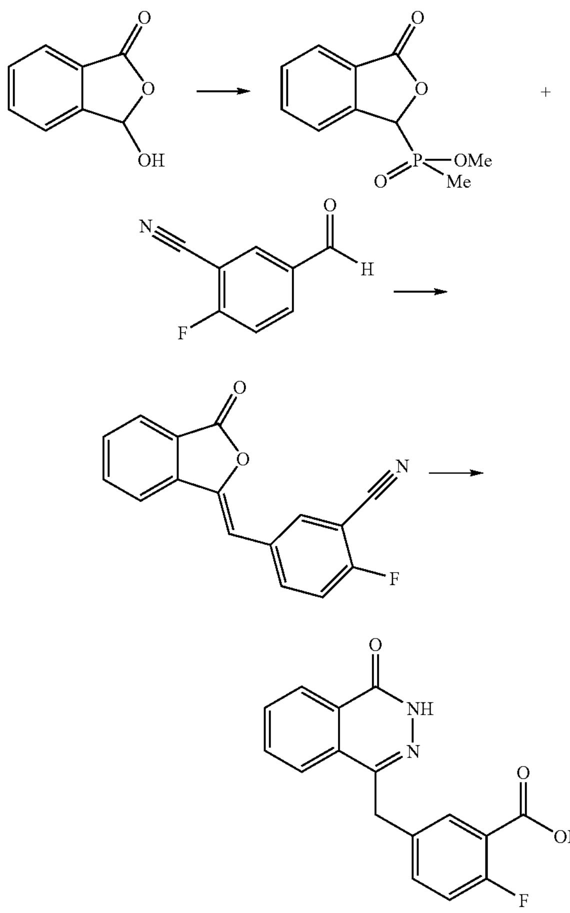
A mixture of 27% sodium methoxide solution in methanol (400 g, 2 mol) and methanol (150 ml) was added dropwise between ambient temperature and 30° C. over 15 minutes to a stirred mixture of phthalide (67 g, 0.5 mol), 3-formylbenzonitrile (65.5 g, 0.5 mol) and ethyl propionate (250 ml), the mixture was stirred at ambient temperature for 40 minutes and at reflux temperature for 1 hour, then it was allowed to cool to ambient temperature. The resulting red solid was collected by filtration, washed with ethyl acetate (2×50 ml) and dissolved in water (1800 ml). The solution was acidified by the addition of acetic acid (60 ml) and the resulting red solid was collected by filtration, washed with water (2×200 ml) and dried in vacuo to give 3-(1,3-dioxoindan-2-yl)benzonitrile (83.2 g) as a dark red solid, m.pt. 179-182° C., m/z $(M+H)^+$. 248, which was used without further purification.

3-(1,3-Dioxoindan-2-yl)benzonitrile (74.18 g, 0.3 mol) was added in portions to a solution of sodium hydroxide (36 g, 0.9 mol) in water (580 ml), the resulting dark red suspension was stirred at reflux temperature for 5 hours, then it was cooled to ambient temperature and washed with ethyl acetate (3×300 ml). The aqueous solution was acidified by the dropwise addition of concentrated hydrochloric acid (110 ml), the mixture was stirred at ambient temperature for 1 hour, then the resulting solid was collected by filtration, washed with water (2×200 ml) and dried in vacuo to give a 1:1 mixture of 3-(1,3-dioxoindan-2-yl)benzoic acid, $(M+H)^+$. 267, and 2-[2-(3-carboxyphenyl)acetyl]benzoic acid, $(M+H)^+$. 285, (69.32 g), which was used without further purification.

The mixture obtained in the previous step (52.8 g) was added to a solution of triethylamine (37.55 g, 0.372 mol) in industrial methylated spirit (500 ml) and the resulting cloudy solution was filtered through a pad of filter-aid to give a clear solution. Hydrazine monohydrate (9.3 g, 0.186 mol) was added in one portion at ambient temperature, the stirred mixture was heated under reflux for 1 hour, then it was concentrated in vacuo to approximately 250 ml and added to a solution of sodium acetate (41 g, 0.5 mol) in water (500 ml). The mixture was brought to pH 7 by the dropwise addition of concentrated hydrochloric acid, then it was stirred at ambient temperature for 3 hours. The resulting solid was collected by filtration, washed with water (50 ml) and dried in vacuo to give a white solid (15.62 g). The combined filtrate and washings were acidified to pH 6 by the addition of hydrochloric acid, then the mixture was stirred at ambient temperature for

3 hours. The resulting solid was collected by filtration, washed with water (50 ml) and dried in vacuo to give a second crop of off-white solid (17.57 g). The combined filtrate and washings from the second crop were readjusted to pH 6 and treated as before to give a third crop of pale orange solid (6.66 g). The three crops were combined to give essentially pure 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A), $(M+H)^{+\cdot}$ 281, δ_H 4.4 (2H, s), 7.2-7.4 (1H, m), 7.5-7.6 (1H, m), 7.7-8.0 (5H, m), 8.1-8.2 (1H, m), 12.6 (1H, s)

b. 2-Fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)benzoic acid (B)



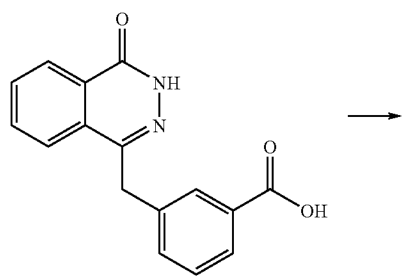
(B)

Dimethyl phosphite (22.0 g, 0.2 mol) was added drop-wise to a solution of sodium methoxide (43.0 g) in methanol (100 ml) at 0° C. 2-Carboxybenzaldehyde (21.0 g, 0.1 mol) was then added portion-wise to the reaction mixture as a slurry in methanol (40 ml), with the temperature kept below 5° C. The resulting pale yellow solution was warmed to 20° C. over 1 hour. Methanesulphonic acid (21.2 g, 0.22 mol) was added to the reaction drop-wise and the resulting white suspension was evaporated in vacuo. The white residue was quenched with water and extracted into chloroform (3×100 ml). The combined organic extracts were washed with water (2×100 ml), dried over $MgSO_4$, and evaporated in vacuo to yield (3-oxo-1,3-dihydro-isobenzofuran-1-yl)phosphonic acid dimethyl ester as a white solid (32.0 g, 95%, 95% purity). This was then used without further purification in the next stage.

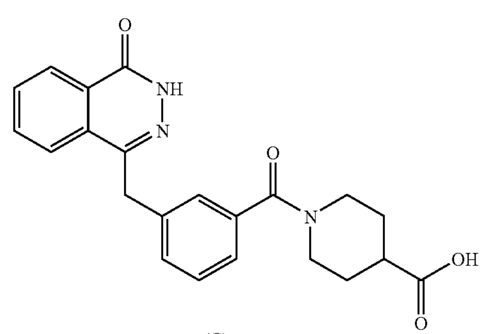
To a mixture of (3-oxo-1,3-dihydro-isobenzofuran-1-yl)phosphonic acid dimethyl ester (35.0 g, 0.14 mol) in tetrahydrofuran (200 ml) and 2-fluoro-5-formylbenzonitrile (20.9 g, 0.14 mol) in tetrahydrofuran (130 ml) was added triethylamine (14 ml, 0.14 mol) drop-wise over 25 min, with the temperature kept below 15° C. The reaction mixture was warmed slowly to 20° C. over 1 hour and concentrated in vacuo. The white residue was slurried in water (250 ml) for 30 minutes, filtered, washed with water, hexane and ether, and dried to yield 2-fluoro-5-(3-oxo-3H-isobenzofuran-1-ylidenemethyl)benzonitrile as a 50:50 mixture of E and Z isomers (37.2 g, 96%); m/z $[M+1]^+$ 266 (98% purity)

To a suspension of 2-fluoro-5-(3-oxo-3H-isobenzofuran-1-ylidenemethyl)benzonitrile in water (200 ml) was added aqueous sodium hydroxide (26.1 g in 50 ml water) solution and the reaction mixture was heated under nitrogen to 90° C. for 30 minutes. The reaction mixture was partially cooled to 70° C., and hydrazine hydrate (100 ml) was added and stirred for 18 hours at 70° C. The reaction was cooled to room temperature and acidified with 2M HCl to pH 4. The mixture was stirred for 10 min and filtered. The resulting solid was washed with water, hexane, ether, ethyl acetate and dried to yield 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid as a pale pink powder (30.0 g, 77%). m/z $[M+1]^+$ 299 (96% purity), δ_H 4.4 (2H, s), 7.2-7.3 (1H, m), 7.5-7.6 (1H, m), 7.8-8.0 (4H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

c. 1-[3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (C)



(A)

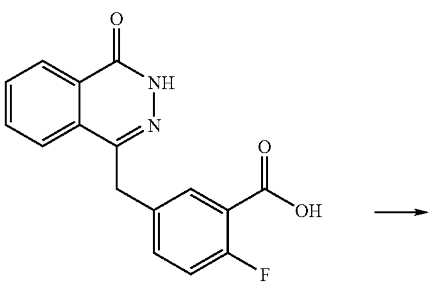


(C)

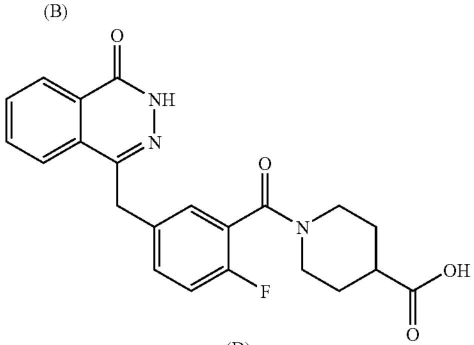
3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)(7.0 g, 0.25 mol), ethyl isonipecotate (5 ml, 0.32 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophospate (HBTU) (12.3 g, 0.32 mol) and N,N,-diisopropylethylamine (10.0 ml, 0.55 mol) were added to dimethylacetamide (40 ml) and stirred for 18 h. Water (100 ml) was added to the reaction mixture and the product was extracted into dichloromethane (4×50 ml). The combined organic layers were washed with water (3×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo to yield an oil. To a solution of the oil in tetrahydrofuran (100 ml) was added 10% aqueous sodium hydroxide solution (20 ml) and the reaction was stirred for 18 hours. The reaction was concentrated, washed with ethyl acetate (2×30 ml) and acidified with 2M HCl to pH 2. The aqueous layer was extracted with dichloromethane (2×100 ml), then the extracts were dried over $MgSO_4$, filtered and evaporated to yield 1-[3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (C) as a yellow solid (7.0 g, 65%), m/z $[M+1]^+$ 392 (96% purity), δ_H 1.3-1.8 (5H, m), 2.8-3.1 (4H, m), 4.4 (2H, s), 7.2-7.3 (1H, m), 7.3-7.4 (1H, m), 7.7-8.0 (5H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

d. 1-[2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (D)



(B)

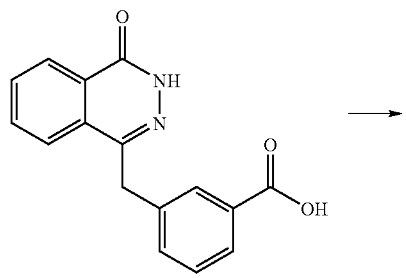


(D)

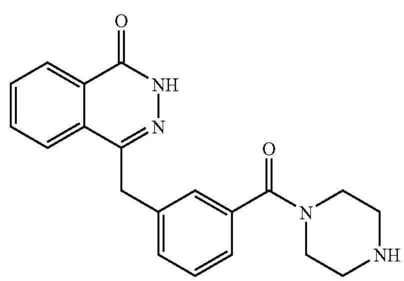
2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl) benzoic acid (B)(3.1 g, 0.14 mol), ethyl isonipecotate (1.7 ml, 0.11 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (5.1 g, 0.13 mol) and N,N,-diisopropylethylamine (10.0 ml, 0.55 mol) were added to dimethylacetamide (15 ml) and stirred for 18 hours. Water (100 ml) was added to the reaction mixture and the product was extracted into dichloromethane (4×50 ml). The combined organic layers were, filtered, washed with water (3×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo to yield an orange oil. The oil was purified by flash chromatography (ethyl acetate) to yield 1-[2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid as the methyl ester (1.5 g, 33%, 96% purity). To a solution of the methyl ester in tetrahydrofuran: water (2:1, 40 ml) was added sodium hydroxide (0.3 g, 0.075 mol) and the reaction was stirred for 18 h. The reaction was concentrated, washed with ethyl acetate (2×20 ml) and acidified with 2M HCl to pH 2. The aqueous layer was extracted with dichloromethane (2×20 ml), and the combined extracts were dried over $MgSO_4$ and evaporated to yield 1-[3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (D) as a yellow solid (0.6 g, 65%), m/z $[M+1]^+$ 392 (96% purity)

EXAMPLE 1

a. Synthesis of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1)



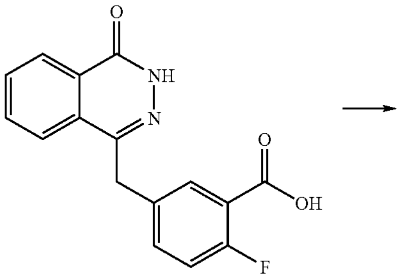
(A)



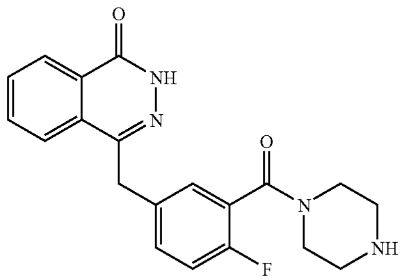
(1)

3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)(5.0 g, 0.17 mol), tert-butyl 1-piperazinecarboxylate (3.9 g, 0.21 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (8.6 g, 0.22 mol) and N,N,-diisopropylethylamine (6.7 ml, 0.38 mol) were added to dimethylacetamide (40 ml) and stirred for 18 hours. Water (100 ml) was added and the reaction mixture was heated to 100° C. for 1 hour. The suspension was cooled to room temperature, filtered and dried to yield a white solid. The solid was dissolved in a solution of 6M HCl and ethanol (2:1, 50 ml) and stirred for 1 hour. The reaction was concentrated, basified with ammonia to pH 9, and the product was extracted into dichloromethane (2×50 ml). The combined organic layers were washed with water (2×50 ml), dried over $MgSO_4$, and evaporated in vacuo to yield 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) as a yellow crystalline solid (4.0 g, 77%); m/z $[M+1]^+$ 349 (97% purity), δ_H 2.6-3.8 (8H, m), 4.4 (2H, s), 7.2-7.5 (4H, m), 7.7 -8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s)

b. Synthesis of 4-[4-Fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2)



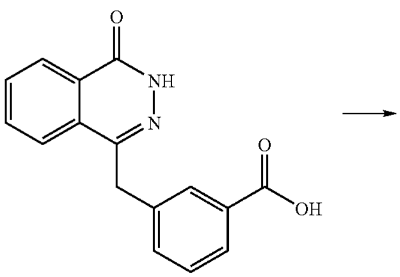
(B)



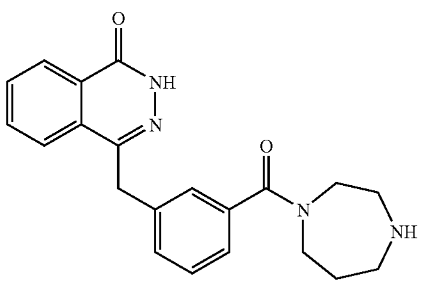
(2)

The synthesis was carried out according to the method described in (a) above using 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (B) to yield 4-[4-fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2) as a white crystalline solid (4.8 g, 76%); m/z $[M+1]^+$ 367 (97% purity), δ_H 2.6-3.8 (8H, m), 4.4 (2H, s), 7.2-7.5 (3H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

c. Synthesis of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3)



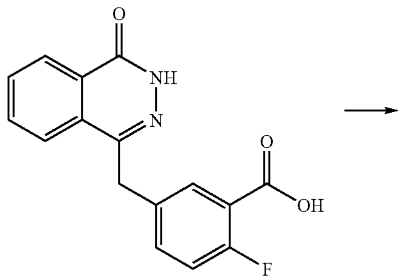
(A)



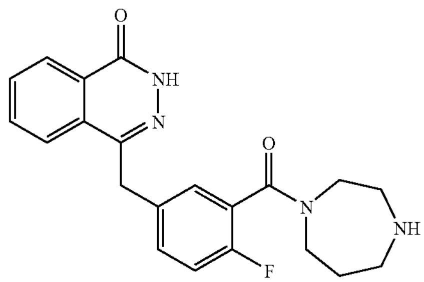
(3)

The synthesis was carried out according to the method described in (a) above using 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) and tert-butyl 1-homopiperazine carboxylate to yield 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) as a grey crystalline solid (5.3 g, 97%); m/z $[M+1]^+$ 363 (97% purity); δ_H 2.6-3.8 (10H, m), 4.4 (2H, s), 7.2-7.5 (4H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

d. Synthesis of 4-[3-([1,4]diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one (4)



(B)

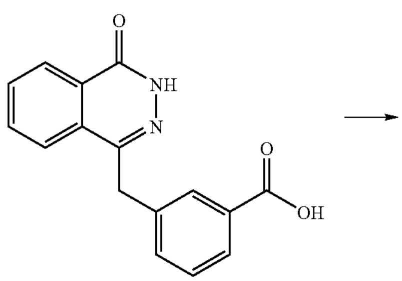


(4)

The synthesis was carried out according to the method described in (a) above using 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (B) and tert-butyl 1-homopiperazinecarboxylate to yield 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (4) as a yellow crystalline solid (5.3 g, 68%); m/z $[M+1]^+$ 381 (97% purity); δ_H 2.6-3.8 (10H, m), 4.4 (2H, s), 7.2-7.5 (3H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

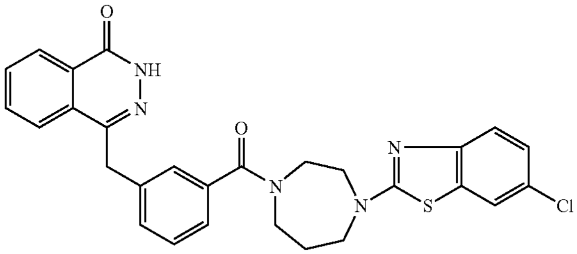
EXAMPLE 2

a. 4-{3-[4-(6-Chlorobenzothiazol-2-yl)-1,4-diazepan-1-ylcarbonyl]benzyl}-1(2H)-phthalazinone



(A)

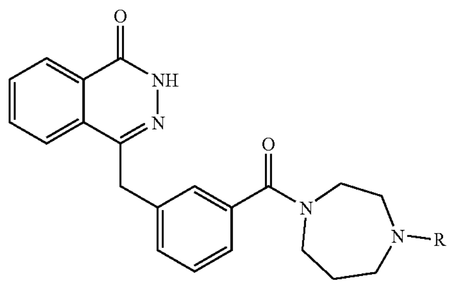
-continued

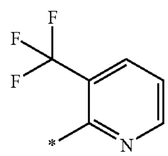
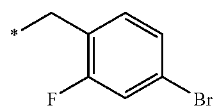
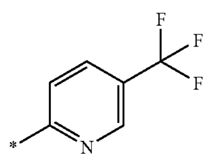
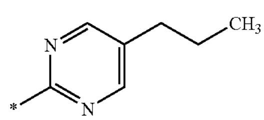
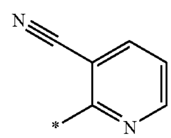
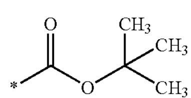


(5)

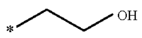
2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (150 mg, 0.47 mmol), diisopropylethylamine (102 mg, 0.8 mmol) and 6-chloro-2-(1,4-diazepan-1-yl)-1,3-benzothiazole (115 mg, 0.43 mmol) were added sequentially at ambient temperature to a stirred solution of 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)(100 mg, 0.36 mmol) in dry dimethylacetamide (1 ml), the mixture was stirred at ambient temperature for 1 hour and allowed to stand at ambient temperature for 16 hours, then it was added drop-wise to stirred cold water (10 ml). After 30 minutes, the resulting solid was collected by filtration, washed with water (2×1 ml) and hexane (1 ml), dried in vacuo and purified using preparative HPLC to give the desired compound (5)(166 mg) as a grey solid; HPLC purity 90%, HPLC Retention time 4.21 minutes; m/z $(M+H)^+$. 530.

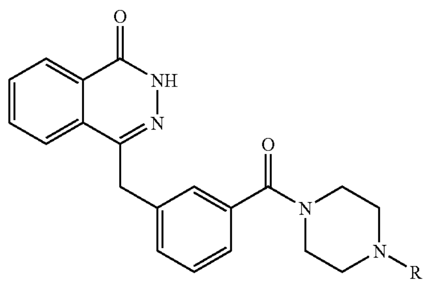
b. The following compounds were synthesised in a manner analogous to that described in (a) above, but using appropriate alternative amine starting materials.

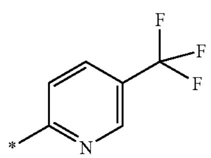
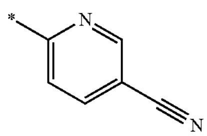
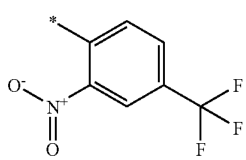
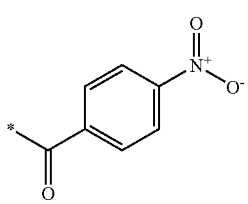
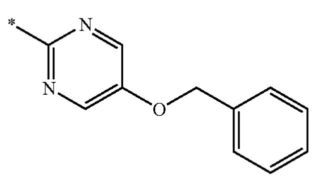


Compound	R	LC RT (minutes)	M + 1	LC PURITY (%)
6		4.18	508	90
7		3.22	551	90
8		4.13	508	90
9		3.95	483	90
10		3.79	465	90
11		3.76	406	90

-continued

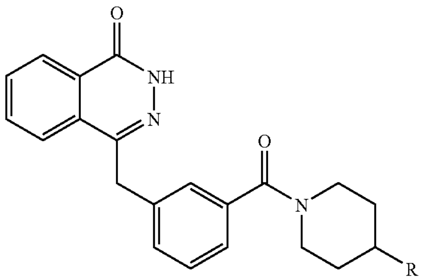
219		2.80	407	90

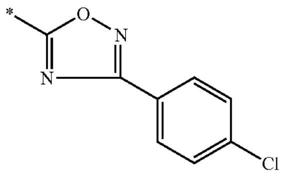
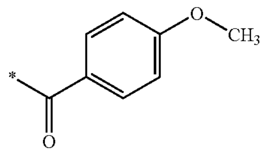
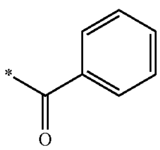
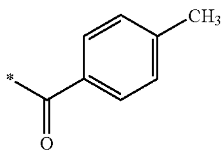


Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
12 (Note 1)		3.56	494	100
13		3.71	451	90
14		4.39	538	90
15		3.66	498	90
16		4.33	533	90

Note 1: 12 did not require purification via preparative scale HPLC—the product from the reaction was essentially pure.

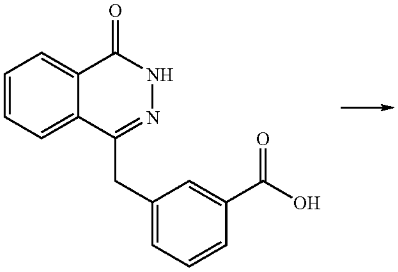
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
17		4.64	526	90
18		3.99	482	90
19		4.00	452	90
20		4.15	466	90

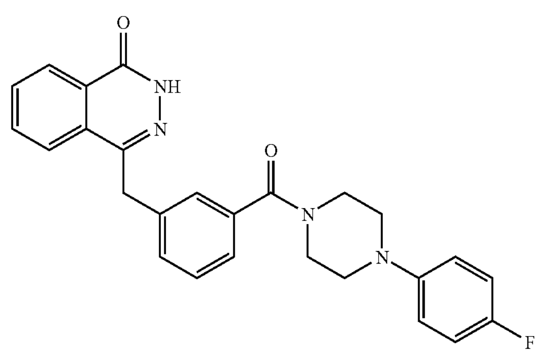
EXAMPLE 3

a. 4-{3-[4-(4-fluorophenyl)piperazin-1-ylcarbonyl]benzyl}-1(2H)-phthalazinone (21)



(A)

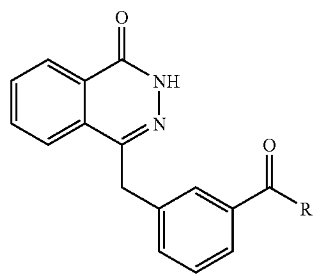
-continued

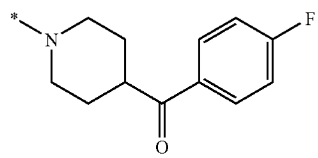
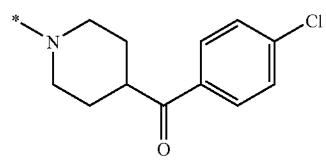
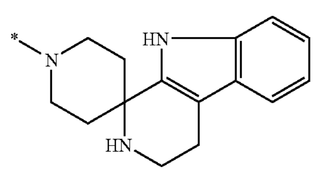
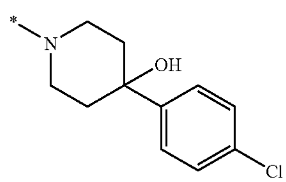


(21)

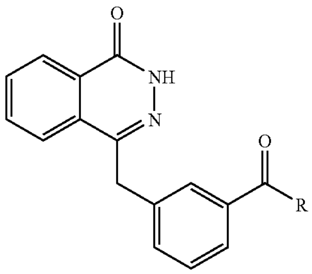
2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (150 mg, 0.47 mmol), diisopropylethylamine (102 mg, 0.8 mmol) and 1-(4-fluorophenyl)piperazine (65 mg, 0.47 mmol) were added sequentially at ambient temperature to a stirred solution of 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)(100 mg, 0.36 mmol) in dry dimethylacetamide (1 ml), the mixture was stirred at ambient temperature for 4 hours and allowed to stand at ambient temperature for 16 hours, then it was added dropwise to stirred cold water (10 ml). After 30 minutes, the resulting solid was collected by filtration, washed with water (2×1 ml) and hexane (1 ml), dried in vacuo and purified using preparative HPLC to give 4-{3-[4-(4-fluorophenyl)piperazin-1-yl-carbonyl]benzyl}-1(2H)-phthalazinone (21) (76 mg) as a cream solid; m/z $(M+H)^{+\cdot}$ 443; HPLC Purity 90%; HPLC Retention time 4.00 minutes.

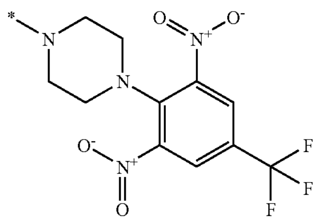
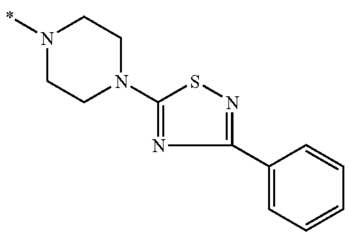
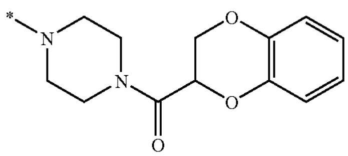
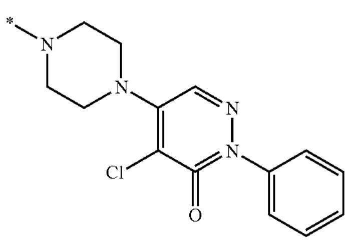
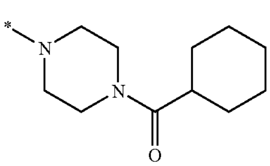
b. The following compounds were synthesised in a manner analogous to that described in (a) above, but using appropriate alternative amine starting materials.



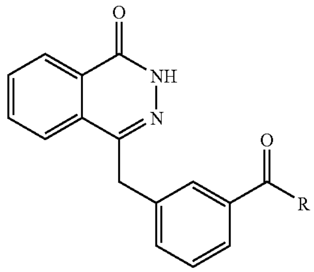
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
22		4.00	470	90
23		4.26	486	90
24		3.18	504	85
25		3.78	473	90

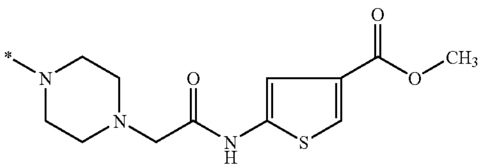
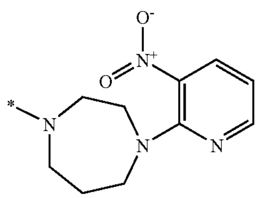
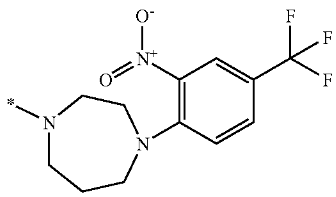
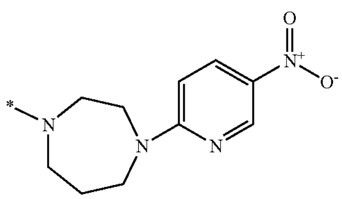
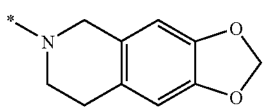
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
26		4.46	583	90
27		4.96	509	90
28		3.73	511	90
29		3.78	553	90
30		3.71	459	90

-continued



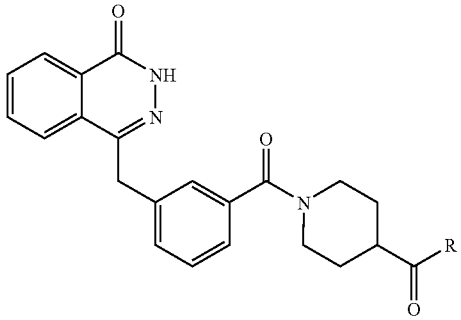
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
31		3.94	546	90
32		3.84	485	90
33		4.37	552	90
34		3.77	485	90
220 (Note 2)		2.89	440	100

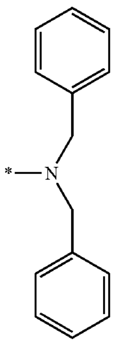
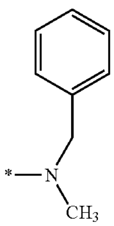
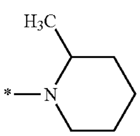
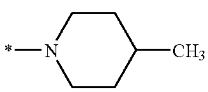
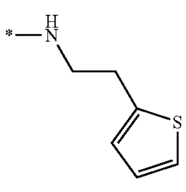
Note 2: 220 did not require purification via preparative scale HPLC—the product from the reaction was essentially pure.

EXAMPLE 4

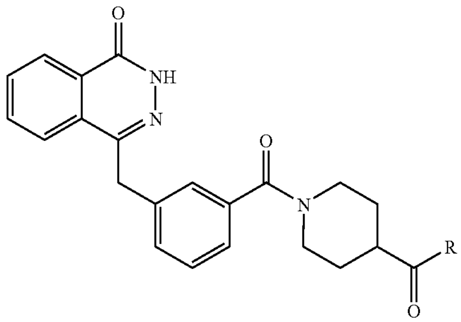
1-[3-(4-Oxo-3,4-dihydro-phthalazin-1-ylmethyl)-benzoyl]-piperidine-4-carboxylic acid (C) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

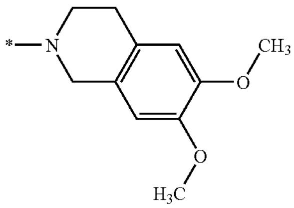
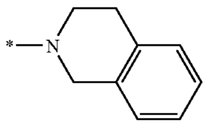
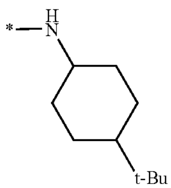
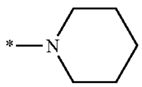
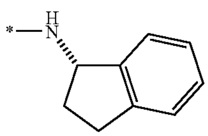
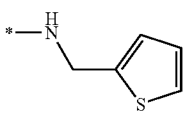
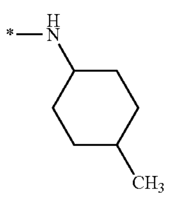
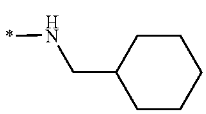
The compounds synthesised are set out below.



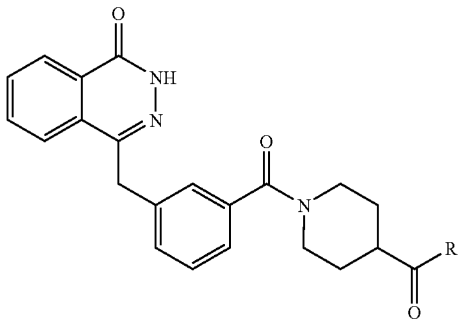
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
35		4.39	572	90
36		3.71	496	90
37		3.63	474	80
38		3.76	474	90
39		3.56	502	90

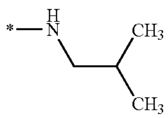
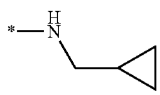
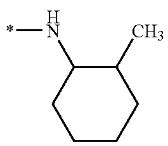
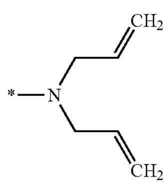
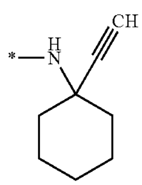
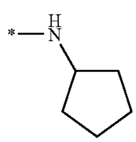
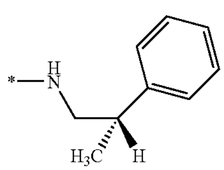
-continued



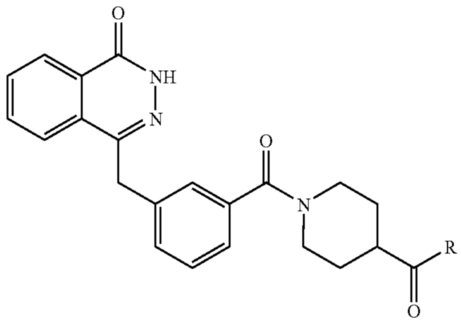
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
40		3.58	568	90
41		3.81	508	90
42		4.39	531	90
43		3.52	460	85
44		3.77	508	90
45		3.59	488	90
46		3.83	488	90
47		3.85	488	90

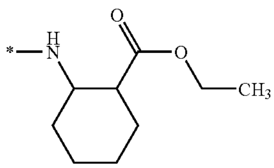
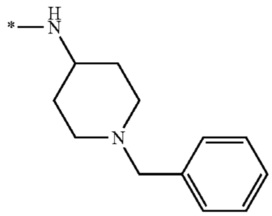
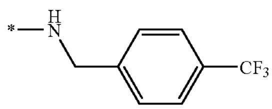
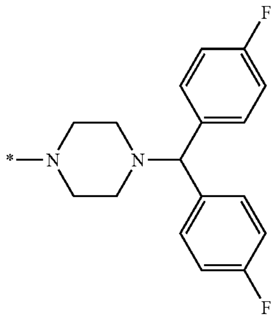
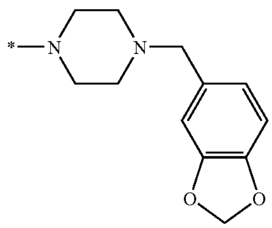
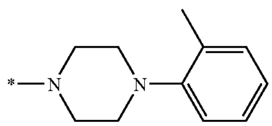
-continued



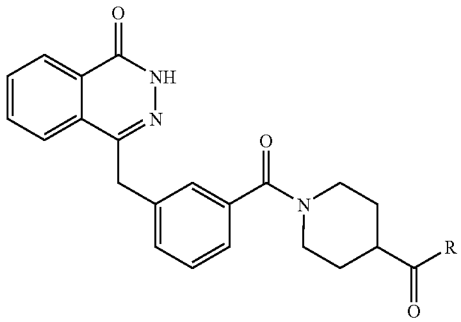
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
48		3.47	448	90
49		3.36	446	90
50		3.77	488	90
51		3.74	472	90
52		3.82	498	90
53		3.52	460	90
54		3.86	510	90

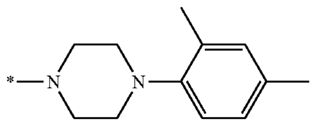
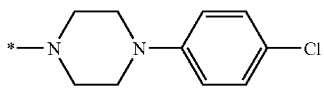
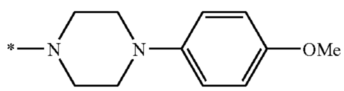
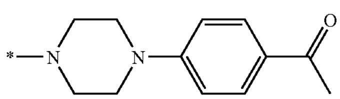
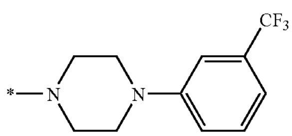
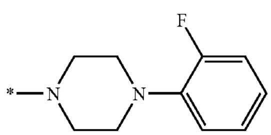
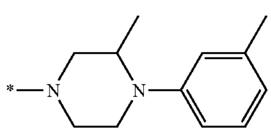
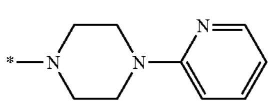
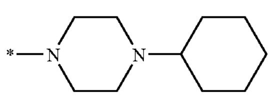
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
55		3.75	546	90
56		3.01	565	90
57		3.93	549	90
373		4.17	663	90
374		3.08	595	90
375		4.16	551	90

-continued

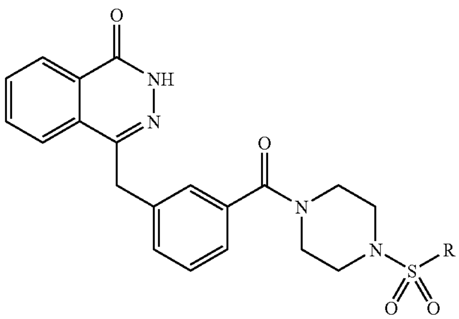


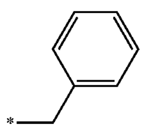
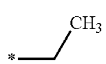
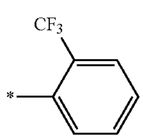
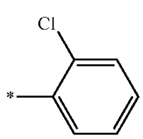
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
376		4.3	565	90
377		4.1	571	90
378		3.64	567	90
379		3.62	579	90
380		4.27	605	90
381		3.89	555	90
382		3.84	565	90
383		2.92	565	90
384		3.02	543	90

EXAMPLE 5

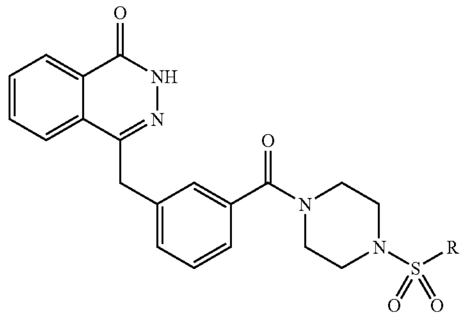
The appropriate sulphonyl chloride (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

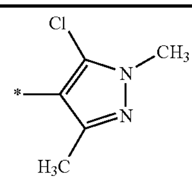
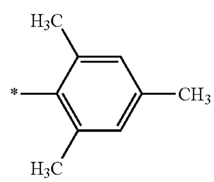
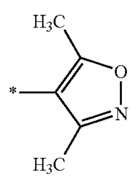
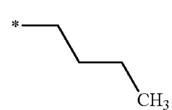
The compound synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
58		3.85	504	90
59		3.44	442	90
60		4.09	558	90
61		3.93	525	90

-continued

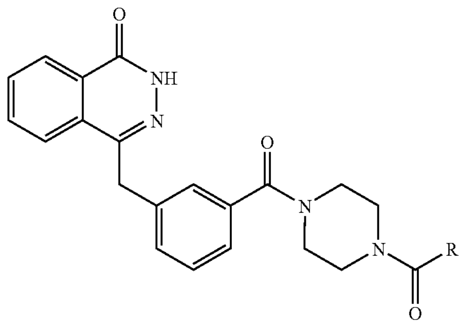


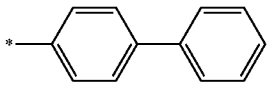
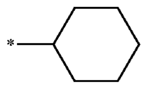
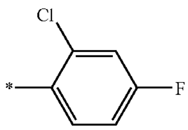
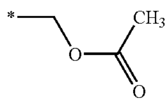
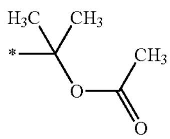
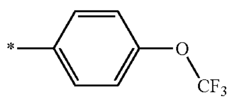
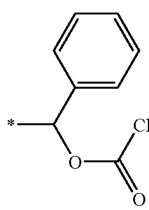
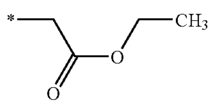
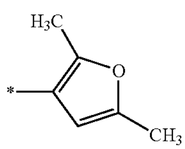
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
62		3.73	543	90
63		4.38	532	90
64		3.76	509	90
65		3.82	470	90

EXAMPLE 6

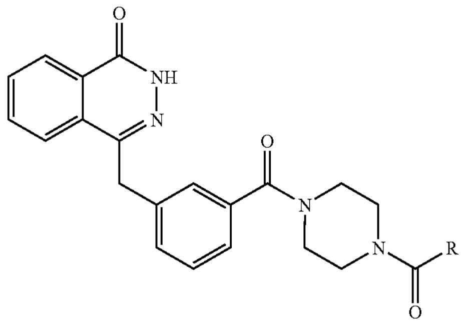
The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

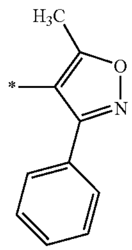
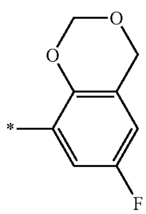
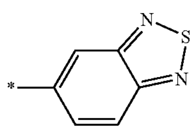
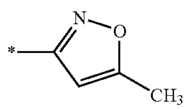
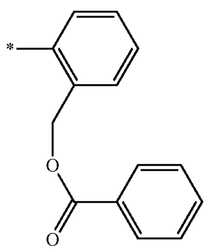
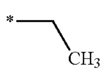
The compounds synthesised are set out below.



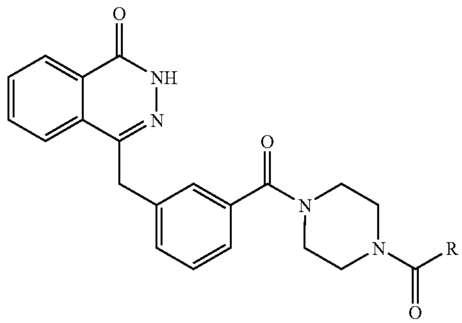
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
66		4.04	530	85
67		3.71	460	90
68		3.69	506	90
69		3.19	450	85
70		3.44	478	90
71		3.94	538	90
72		3.68	526	90
73		3.6	464	90
74		3.63	472	90

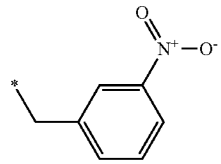
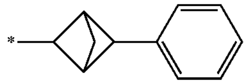
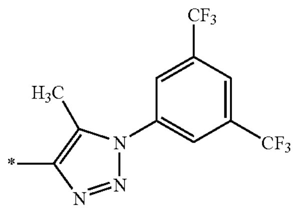
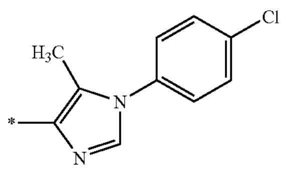
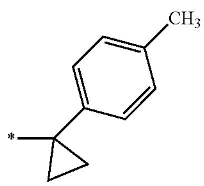
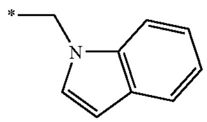
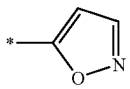
-continued



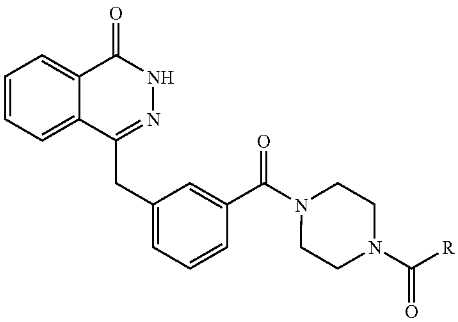
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
75		3.73	535	80
76		3.49	530	90
77		3.5	512	85
78		3.52	459	90
79		4.01	588	90
80		4.05	406	90

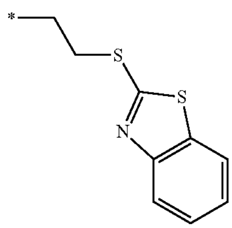
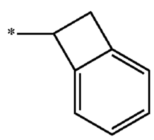
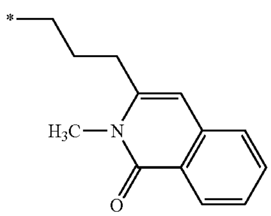
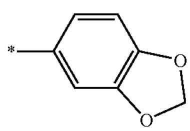
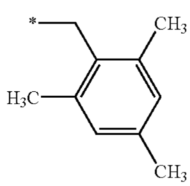
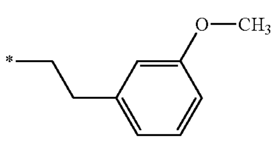
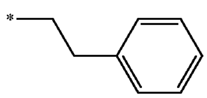
-continued



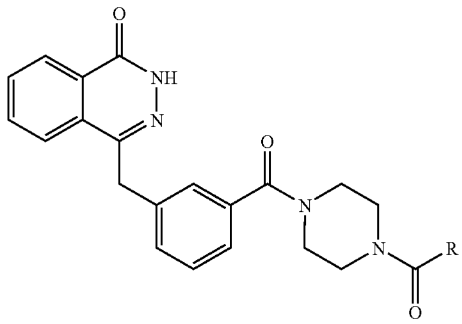
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
81		3.84	513	90
82		4.07	520	90
83		4.41	671	90
84		3.62	582	90
85		3.82	508	90
86		3.81	507	90
87		3.33	445	90

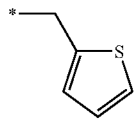
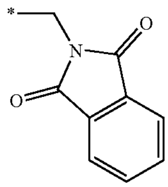
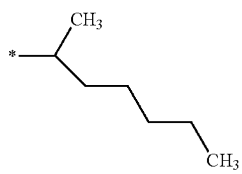
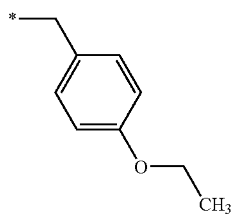
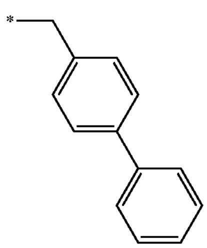
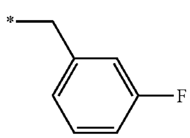
-continued



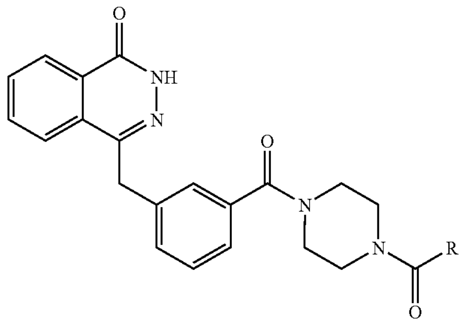
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
88		4.08	571	90
89		3.67	480	90
90		3.54	577	90
91		3.49	498	90
92		4.04	510	90
93		3.75	512	90
94		3.67	482	90

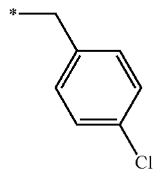
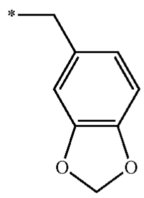
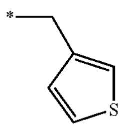
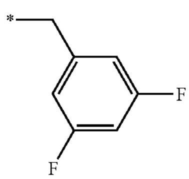
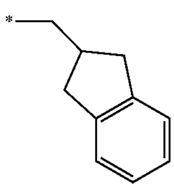
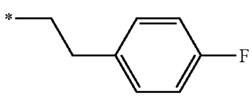
-continued



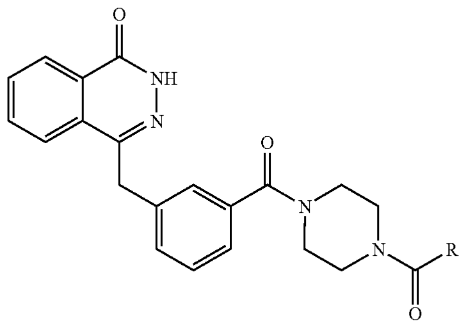
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
95		3.54	474	90
96		3.52	537	90
97		4.13	475	90
98		3.8	512	85
99		4.09	544	90
100		3.63	486	90

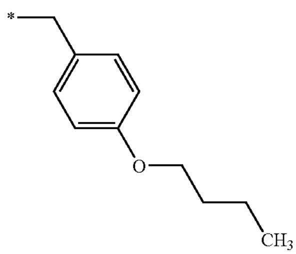
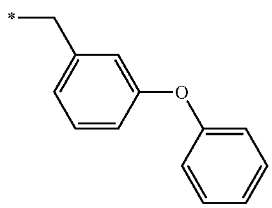
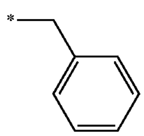
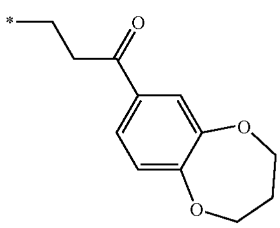
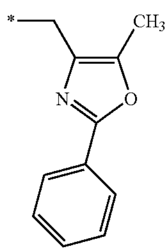
-continued



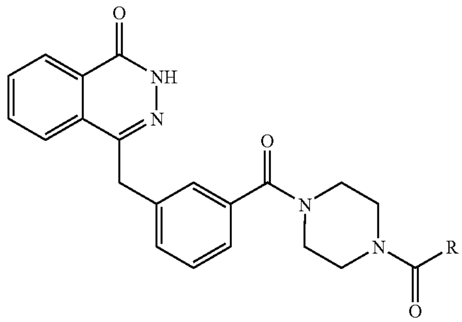
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
101		3.91	502	90
102		3.61	511	90
103		3.57	474	90
104		3.67	504	90
105		4.02	508	90
106		3.81	500	90

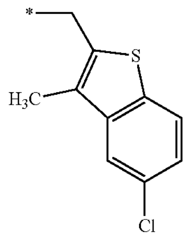
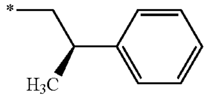
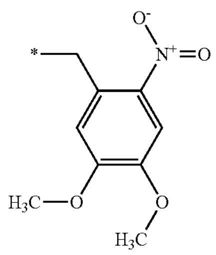
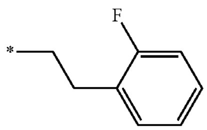
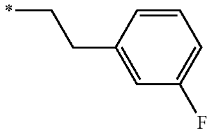
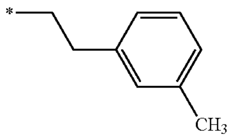
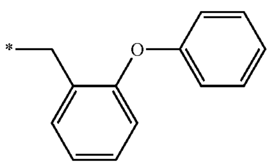
-continued



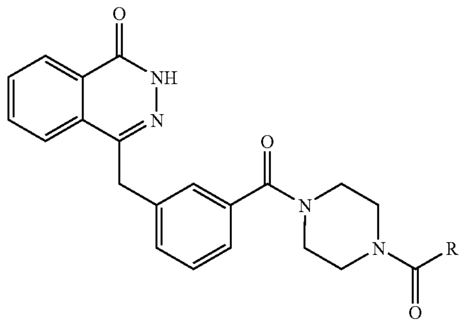
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
107		4.11	540	90
108		4.19	560	90
109		3.61	468	90
110		3.69	582	90
111		3.85	549	85

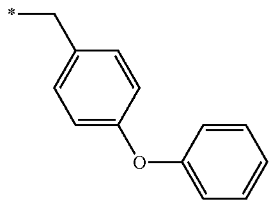
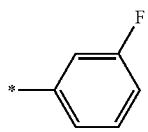
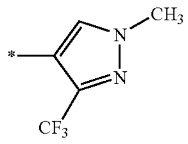
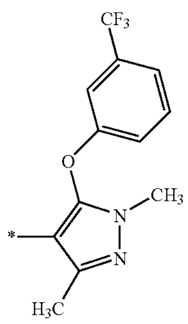
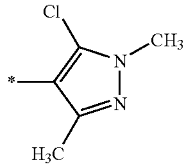
-continued



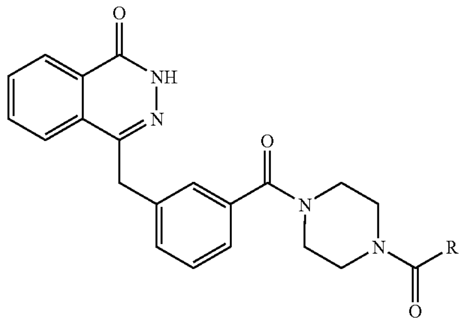
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
112		4.37	573	90
113		3.84	496	90
114		3.62	573	90
115		3.72	500	90
116		3.8	500	85
117		3.9	496	90
118		4.03	560	90

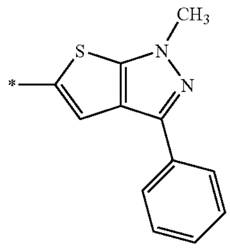
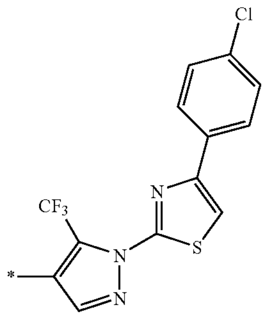
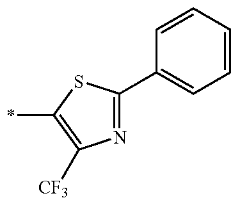
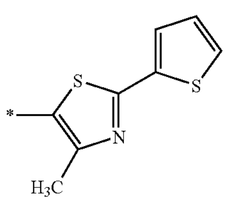
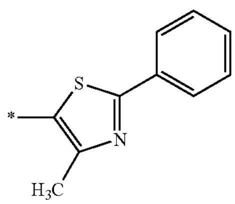
-continued



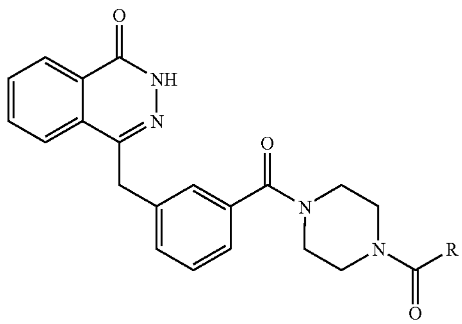
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
119		4.16	560	90
120		4.71	472	80
121		3.47	526	90
122		3.78	632	90
123		3.27	506	90

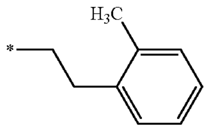
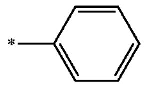
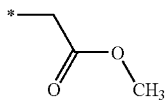
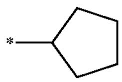
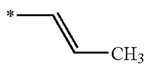
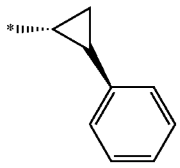
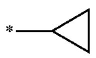
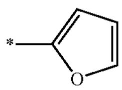
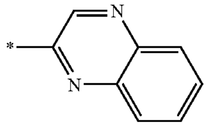
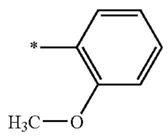
-continued



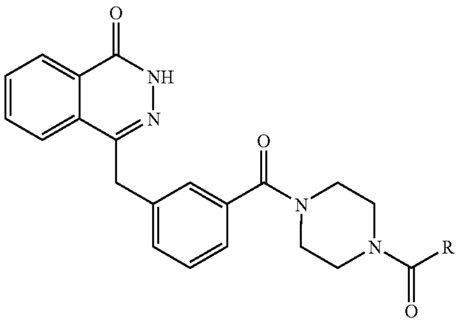
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
124		3.92	590	90
125		4.76	706	90
126		4.27	605	90
127		3.71	557	90
128		3.98	551	90

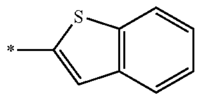
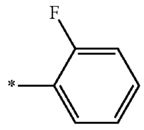
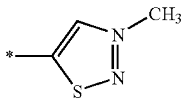
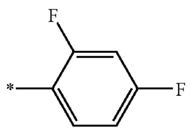
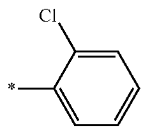
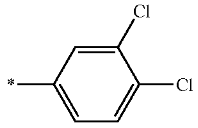
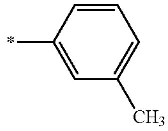
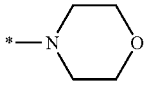
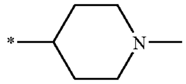
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
129		3.9	496	90
130		3.57	454	90
131		3.21	450	90
132		3.61	446	90
133		3.39	418	85
134		3.81	494	90
135		3.31	418	90
136		3.38	444	90
137		3.56	506	85
138		3.48	484	90

-continued

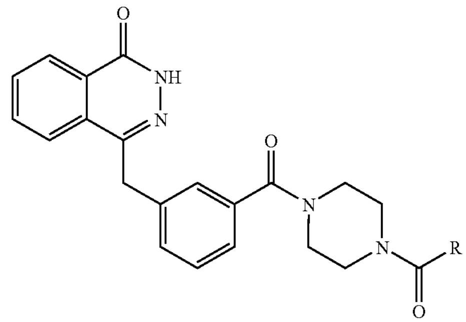


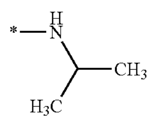
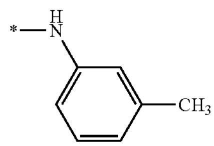
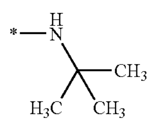
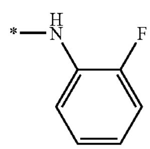
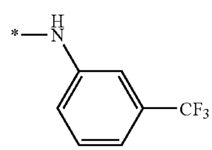
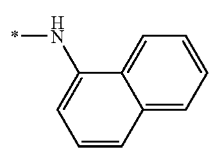
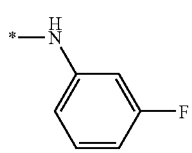
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
139		3.84	510	90
140		3.56	472	90
141		3.34	476	90
142		3.56	490	90
143		3.53	488	90
144		3.99	523	90
145		3.7	468	90
192	*—CH_3	3.11	392	90
350		3.16	462	90
351		2.77	474	90

EXAMPLE 7

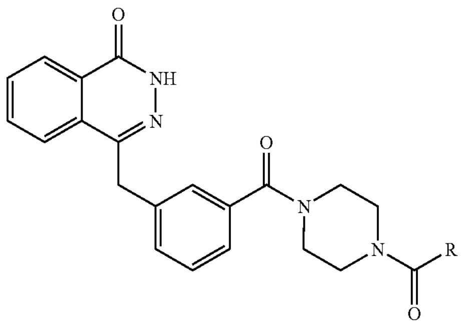
The appropriate isocyanate (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

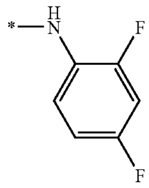
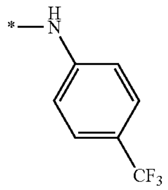
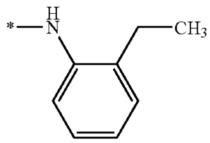
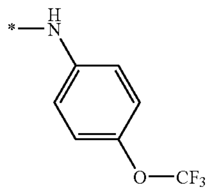
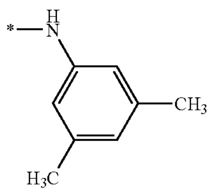
The compounds synthesised are set out below.



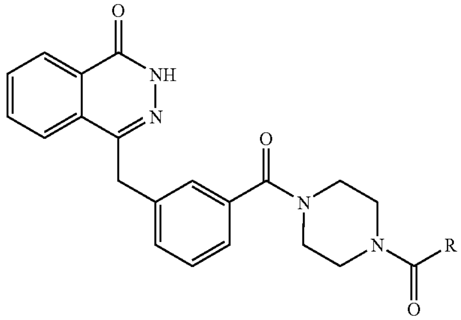
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
146		3.34	435	85
147		3.76	483	90
148		3.49	449	90
149		3.57	487	90
150		4.03	537	90
151		3.77	519	85
152		3.72	487	90

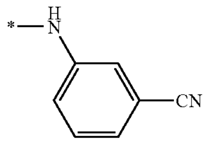
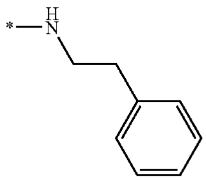
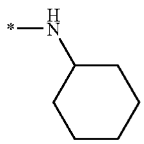
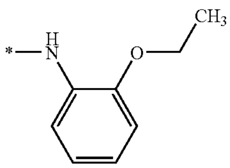
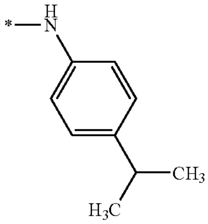
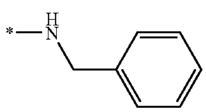
-continued



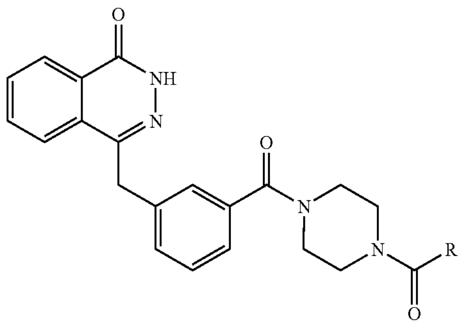
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
153		3.57	505	90
154		4.08	537	80
155		3.68	497	85
156		4.03	553	90
157		3.93	497	90

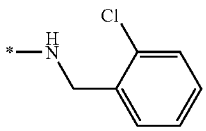
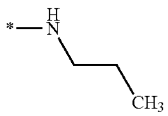
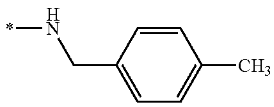
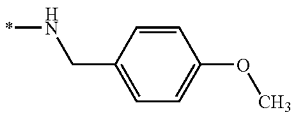
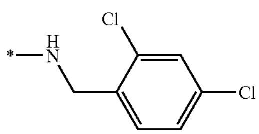
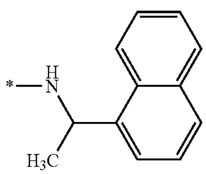
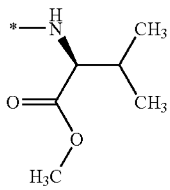
-continued



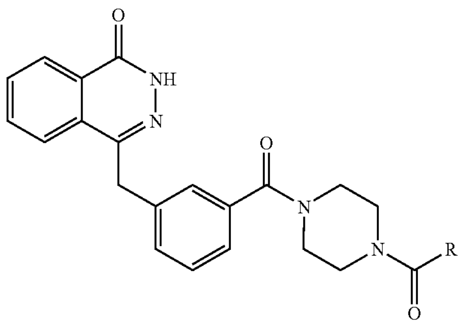
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
158		3.62	494	90
159		3.68	497	90
160		3.73	475	90
161		3.9	513	90
162		4.11	511	90
163		3.58	483	90

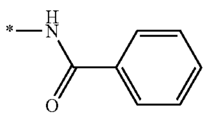
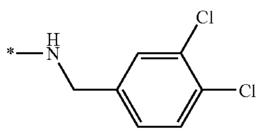
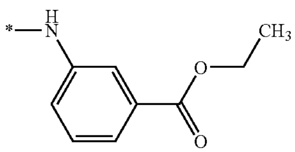
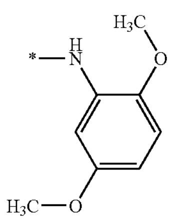
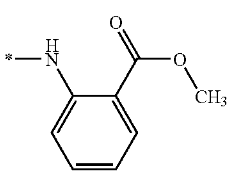
-continued



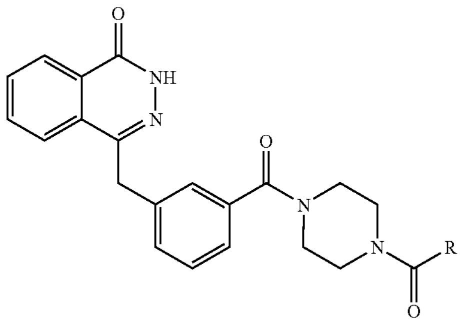
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
164		3.71	517	90
165		3.34	435	85
166		3.71	497	90
167		3.56	513	90
168		4.04	552	90
169		4	547	90
170		3.54	507	90

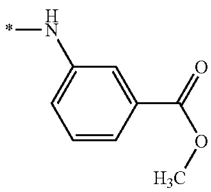
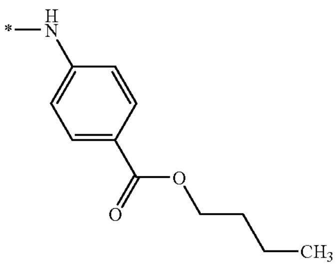
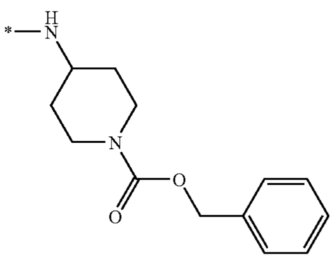
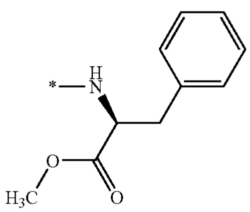
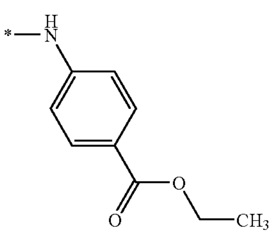
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
171		3.42	497	90
172		3.95	552	90
173		3.79	541	85
174		3.66	529	90
175		3.92	527	85

-continued

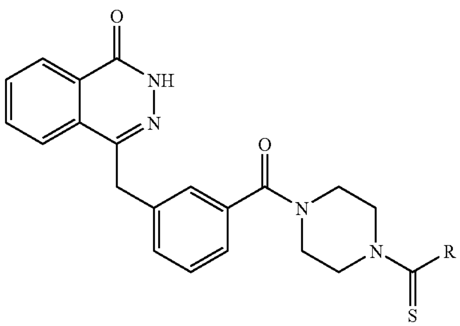


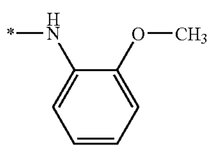
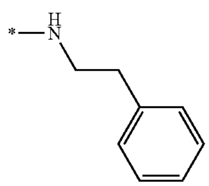
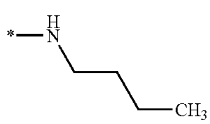
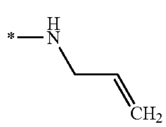
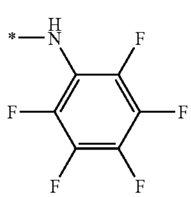
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
176		3.62	527	90
177		4.28	569	90
178		3.81	610	90
352		3.73	555	90
353		3.79	541	90

EXAMPLE 8

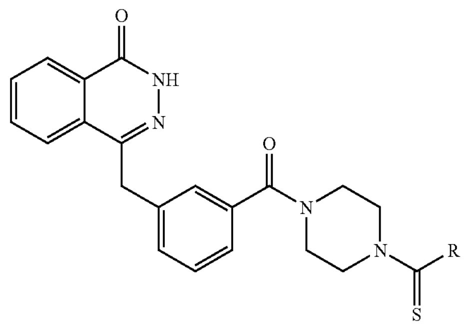
The appropriate isothiocyanate (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

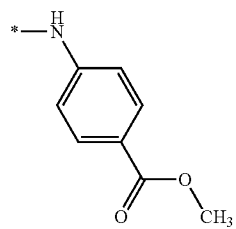
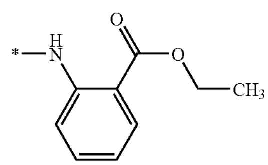
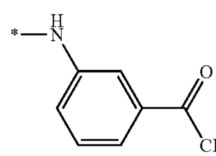
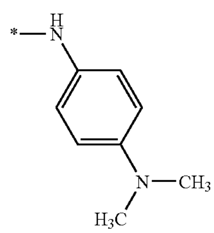
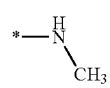
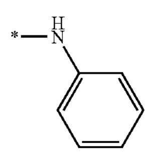
The compounds synthesised are set out below.



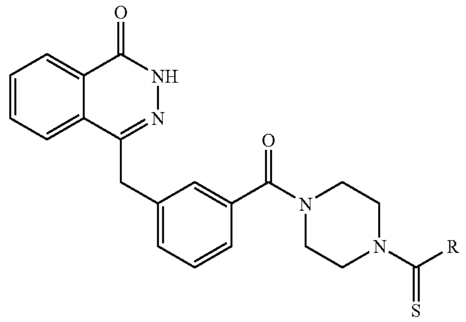
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
179		3.68	515	90
180		4.05	513	90
181		3.94	465	90
182		3.55	449	90
183		4.21	575	90

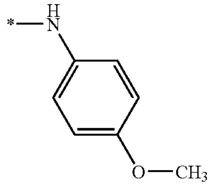
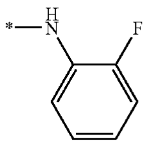
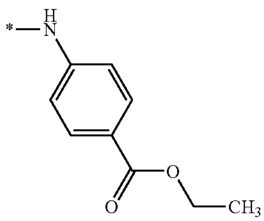
-continued



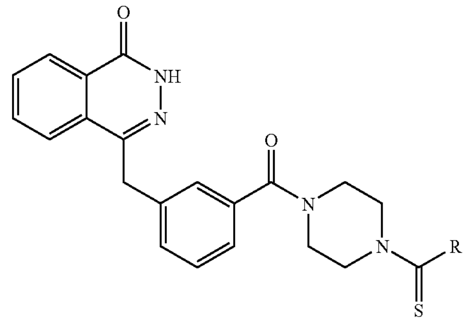
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
184		3.79	543	90
185		4.28	557	85
186		3.63	527	90
187		3.18	528	90
188		3.32	423	90
189		3.69	485	80

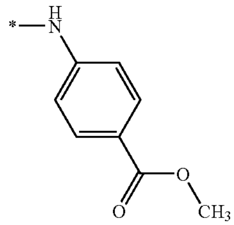
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
190		3.68	515	90
191		3.72	503	90
354		4.67	542	90

-continued

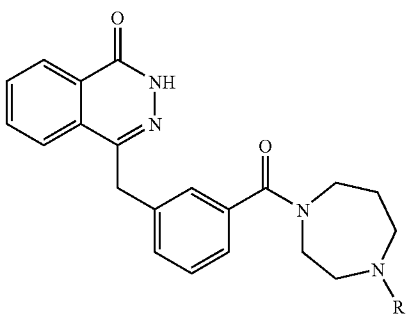
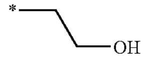


Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
355		3.96	543	90

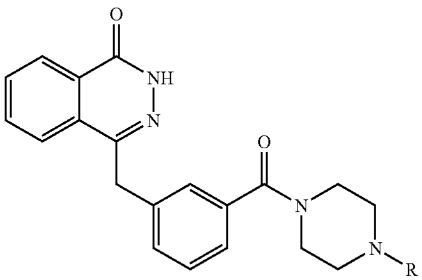
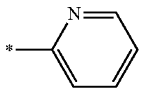
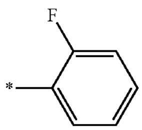
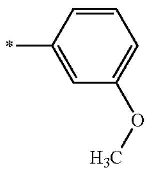
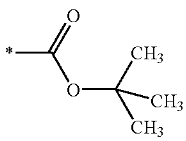
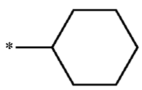
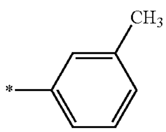
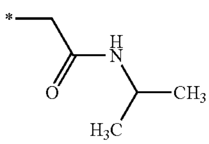
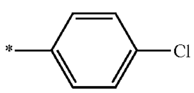
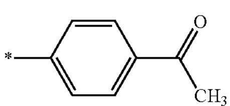
EXAMPLE 9

3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

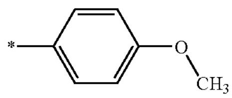
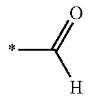
The compounds synthesised are set out below.

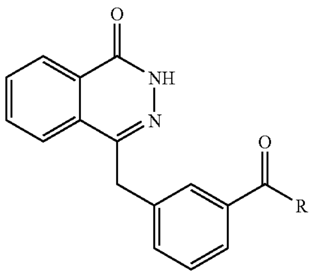
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
				
193	*—CH_3	2.72	378	90
221		2.77	407	90

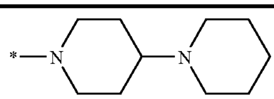
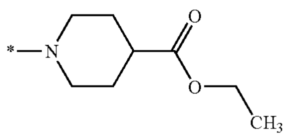
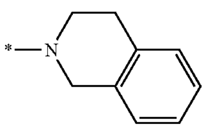
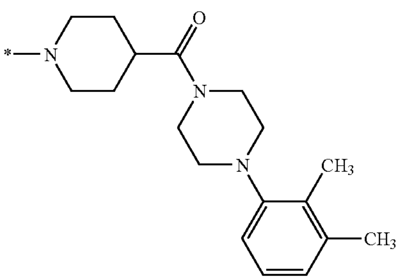
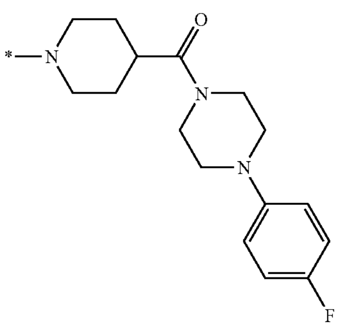
-continued

Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
				
194		2.96	427	90
195		4.08	444	90
196		3.9	456	95
197		3.83	450	95
198		2.98	432	90
199		4.17	440	90
200		2.9	449	90
201		4.31	460	90
202		3.63	468	90

-continued

Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
203		3.78	456	90
204		3.08	378	90

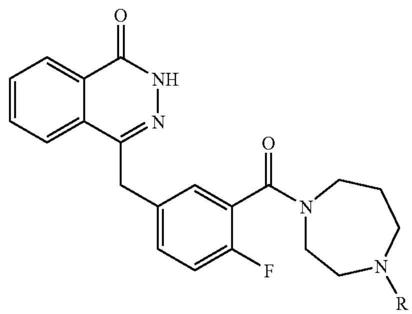
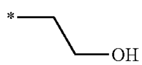
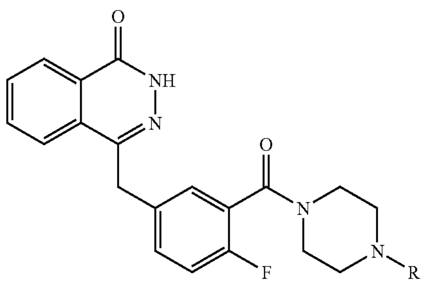
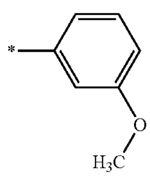
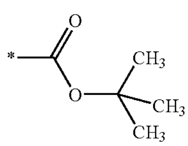
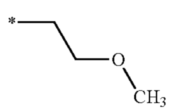


Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
205		2.88	432	90
206		3.61	421	95
207		3.96	397	90
356		4.34	564	90
357		3.84	554	90

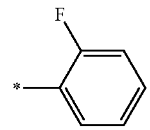
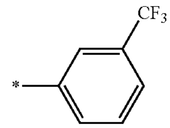
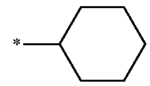
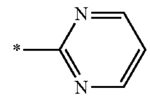
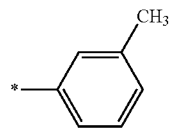
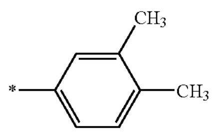
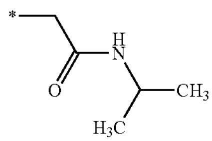
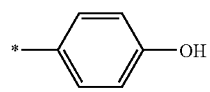
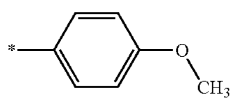
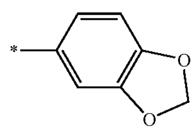
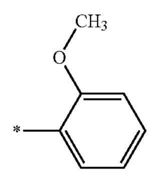
EXAMPLE 10

2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl) benzoic acid (B) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

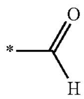
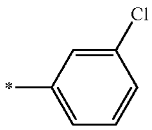
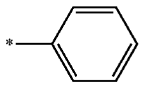
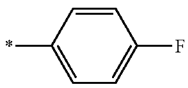
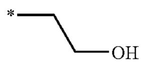
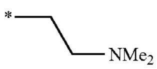
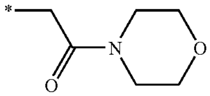
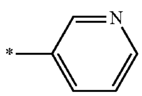
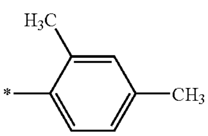
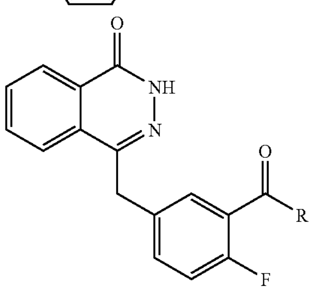
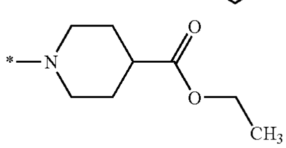
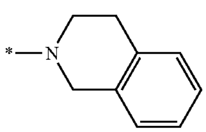
The compounds synthesised are set out below.

Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
				
208	*—CH_3	2.8	396	90
359		2.78	425	85
				
209		3.9	456	95
210		3.97	467	90
211		2.84	426	90
212	*—CH_3	3.46	368	90

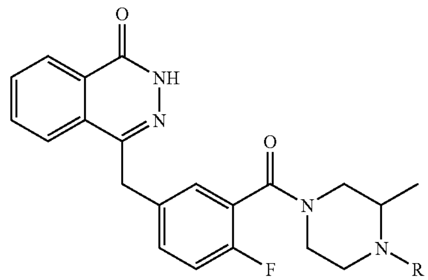
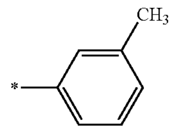
-continued

Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
222		5.07	461	90
223		4.46	511	90
224		2.96	499	90
225		3.55	445	85
226		4.19	457	90
227		4.34	471	90
228		2.88	466	90
229		4.23	477	90
230		4.03	473	90
231		3.07	500	90
232		3.92	473	90

-continued

Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
233		4.55	471	90
234		4.32	477	90
235		3.94	443	90
236		4.03	461	90
237		2.69	411	90
238		2.76	438	90
239		2.77	494	90
240		2.91	444	90
358		4.59	472	90
				
213		3.81	439	90
214		3.95	415	90

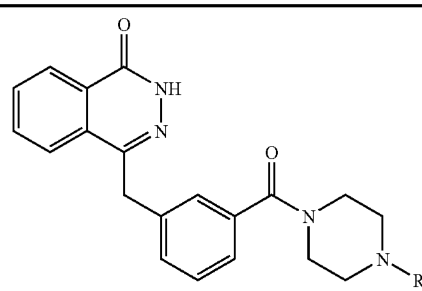
-continued

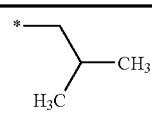
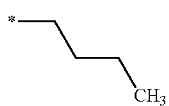
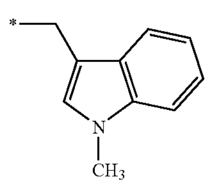
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
				
360		4.08	472	90

EXAMPLE 11

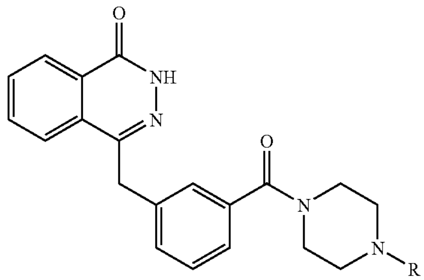
An appropriate aldehyde (0.2 mmol) and 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1)(0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

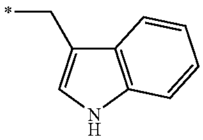
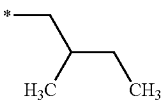
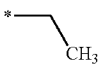
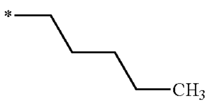
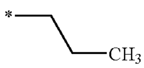
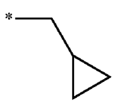
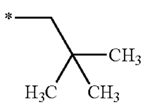
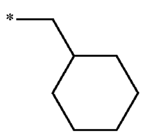
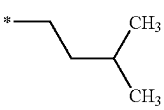
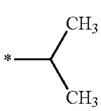
The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
215		2.89	406	90
216		2.91	406	90
217		3.16	493	90

-continued

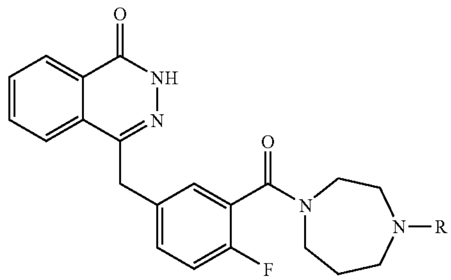


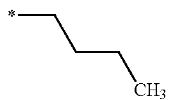
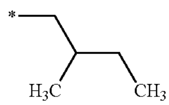
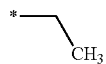
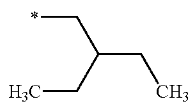
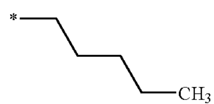
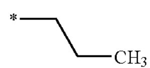
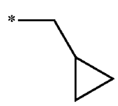
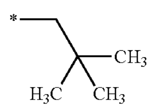
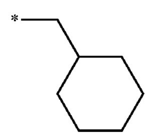
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
218		3.09	479	90
361		2.98	419	90
362		2.74	377	90
363		3.04	419	90
364		2.82	391	85
365		2.84	403	90
366		2.96	419	90
367		3.15	445	90
368		3.04	419	90
369		2.75	391	85

EXAMPLE 12

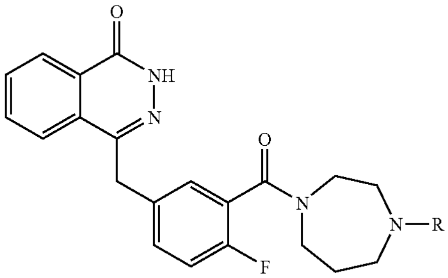
An appropriate aldehyde (0.2 mmol) and 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (4)(0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

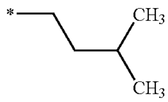
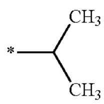
The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
241		2.9	437	90
242		3.05	451	90
243		2.84	409	90
244		3.12	465	90
245		3.16	451	90
246		2.86	423	90
247		2.89	435	90
248		3.04	451	90
249		3.23	477	90

-continued

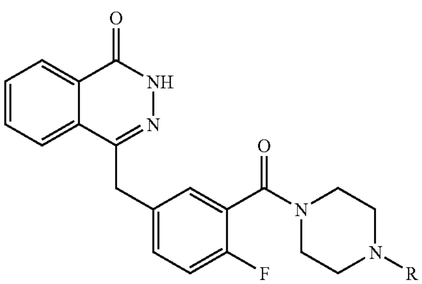


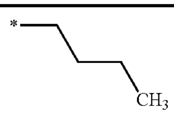
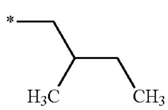
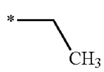
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
250		3.09	451	90
370		2.80	423	90

EXAMPLE 13

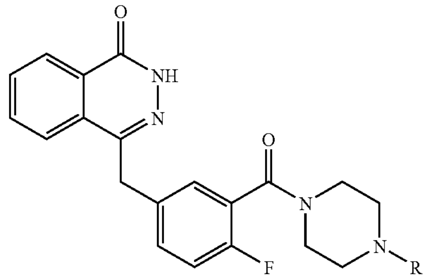
An appropriate aldehyde (0.2 mmol) and 4-[4-fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2)(0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

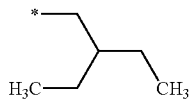
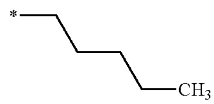
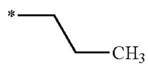
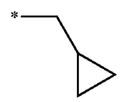
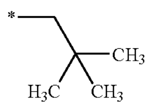
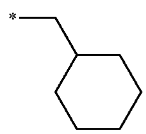
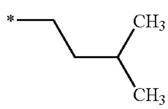
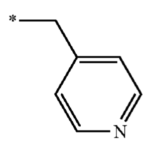
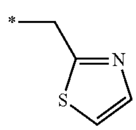
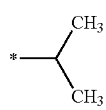
The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
251		2.97	423	90
252		3.06	437	90
253		2.8	395	90

-continued

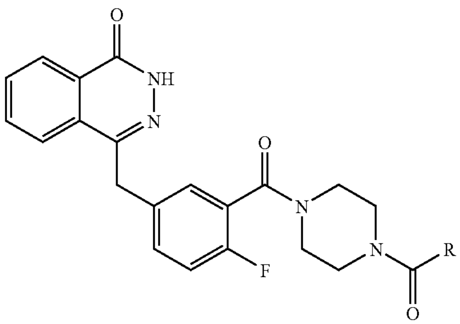


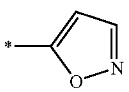
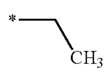
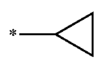
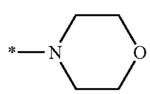
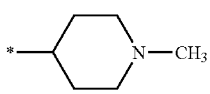
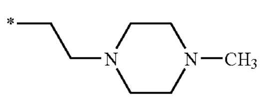
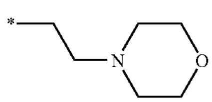
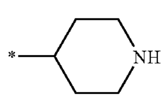
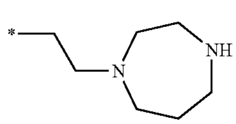
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
254		3.11	451	90
255		3.09	437	90
256		2.87	409	90
257		2.89	421	90
258		3.01	437	85
259		3.14	463	90
260		3.08	437	90
261		2.83	458	90
262		3.04	464	90
263		2.79	409	90

EXAMPLE 14

The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[4-fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

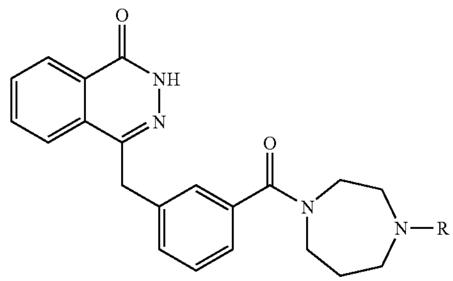


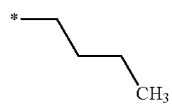
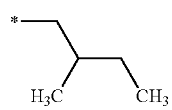
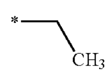
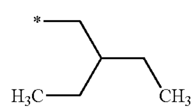
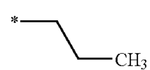
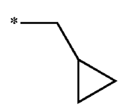
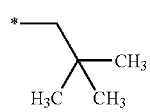
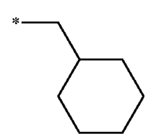
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
264	*—CH_3	3.89	409	90
265		4.17	462	85
266		4.04	423	90
267		4.03	417	90
268		3.19	480	90
269		2.84	492	90
270		2.71	521	90
271		2.83	508	90
272		2.86	478	90
273		2.63	521	90

EXAMPLE 15

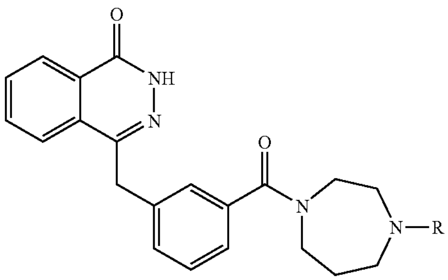
An appropriate aldehyde (0.2 mmol) and 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3)(0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

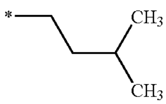
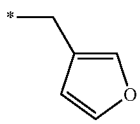
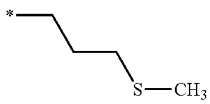
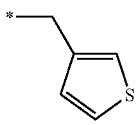
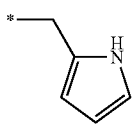
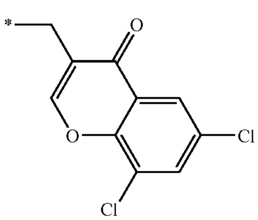
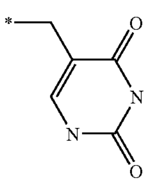
The compounds synthesised are set out below.



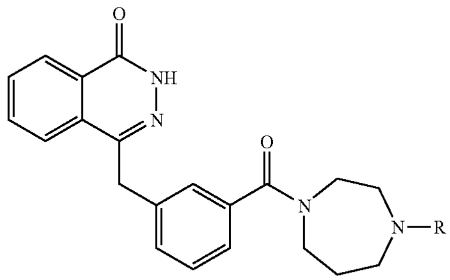
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
274		2.92	419	90
275		3.04	433	90
276		2.78	391	90
277		3.09	433	90
278		2.86	405	90
279		2.88	417	90
280		2.99	433	90
281		3.11	459	90

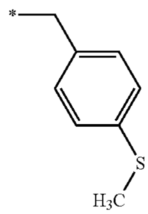
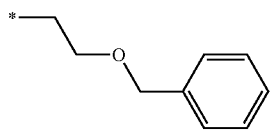
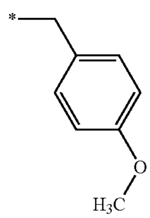
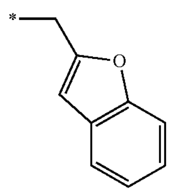
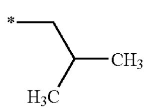
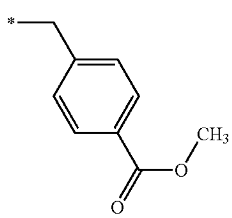
-continued



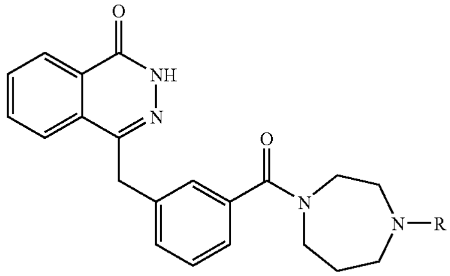
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
282		3.06	433	90
283		2.93	443	90
284		2.92	451	90
285		2.99	459	90
286		2.94	441	90
287		3.36	589	85
288		2.72	487	85

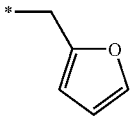
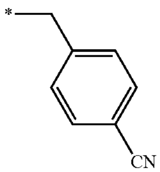
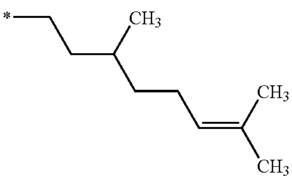
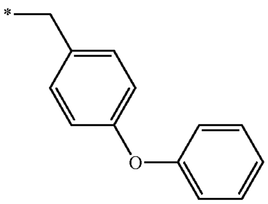
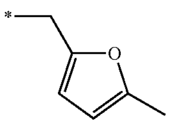
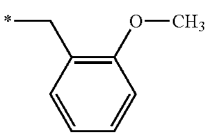
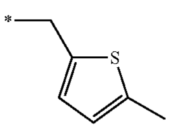
-continued



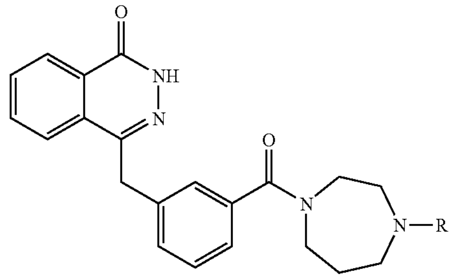
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
289		3.24	499	90
290		3.14	497	90
291		3.9	483	85
292		3.22	493	90
293		2.91	419	90
294		3.08	511	90

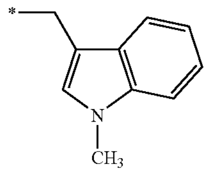
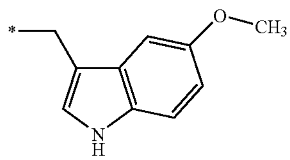
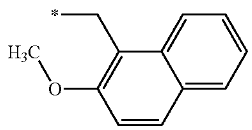
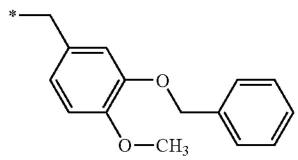
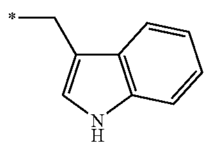
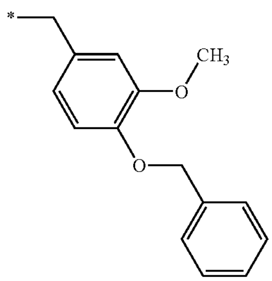
-continued



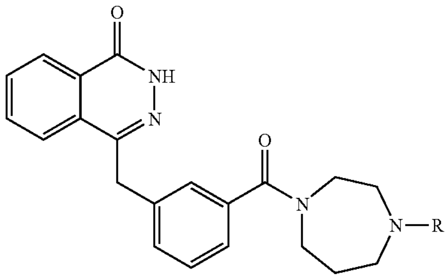
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
295		2.92	443	90
296		3.03	478	90
297		3.48	501	90
298		3.39	545	90
299		3.06	456	90
300		3.8	483	90
301		3.08	472	90

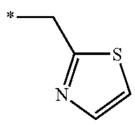
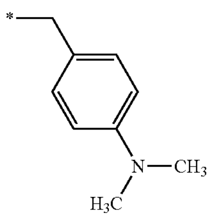
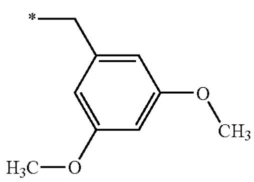
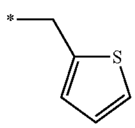
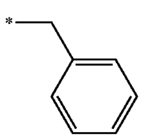
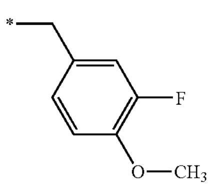
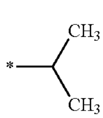
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
302		3.22	506	90
303		3.13	522	90
304		3.29	533	90
305		3.39	589	90
306		3.07	492	90
307		3.41	589	90

-continued

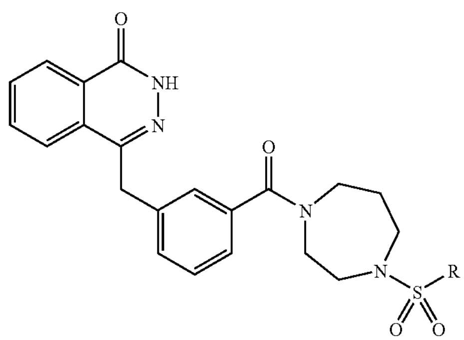


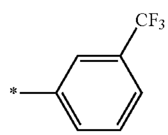
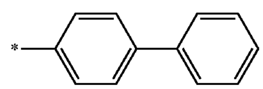
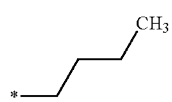
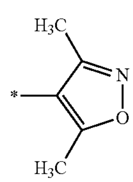
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
308		2.87	460	85
309		3.06	496	90
310		3.13	513	90
311		2.96	459	90
312		2.98	453	90
313		3.09	501	90
314		2.76	405	90

EXAMPLE 16

The appropriate sulphonyl chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

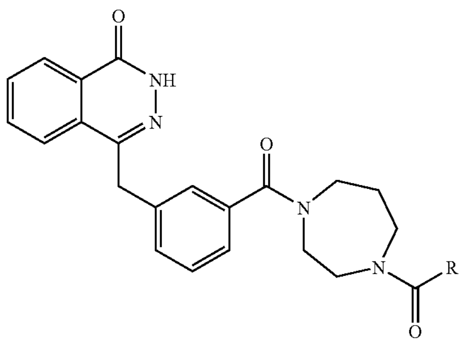


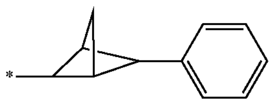
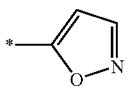
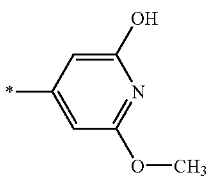
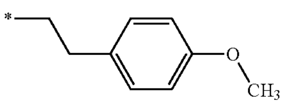
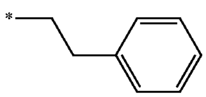
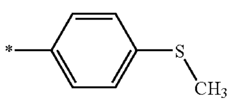
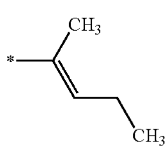
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
315		4.15	571	90
316		4.36	579	90
317		3.68	483	90
371		3.71	523	90

EXAMPLE 17

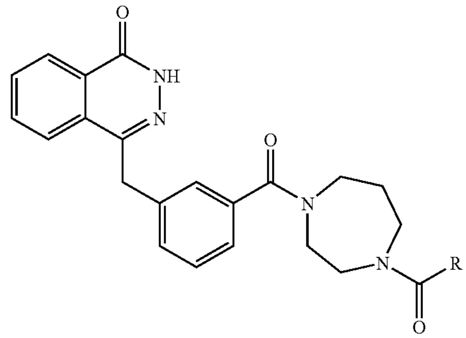
The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

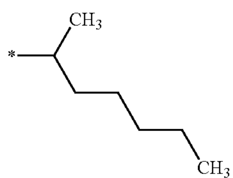
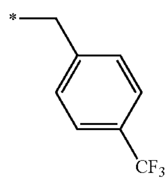
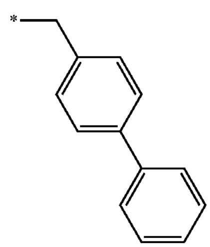
The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
318		3.84	533	90
319		3.23	458	90
320		3.63	532	90
321		3.61	525	90
322		3.63	495	90
323		3.62	513	90
324	*—CN	3.16	415	90
325		3.52	459	90

-continued

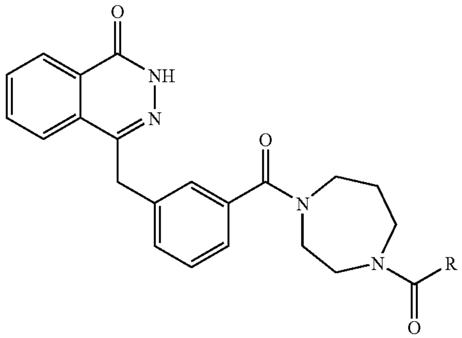


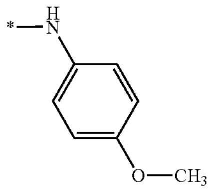
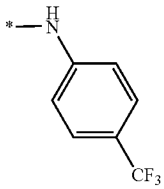
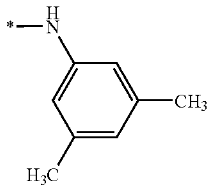
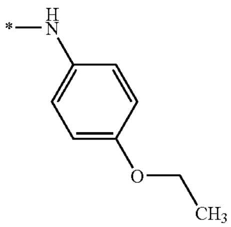
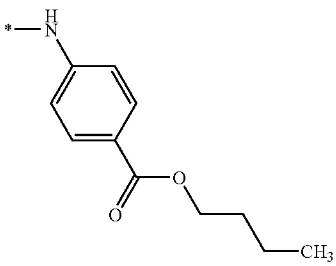
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
326		3.95	489	90
327		3.89	549	90
328		4.00	557	90

EXAMPLE 18

The appropriate isocyanate (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

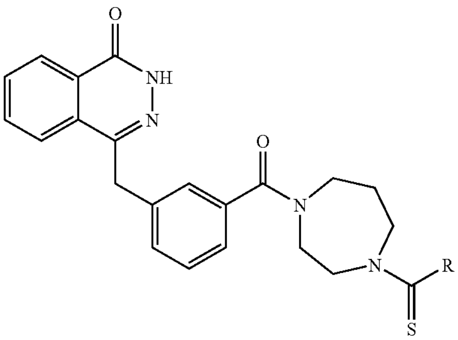


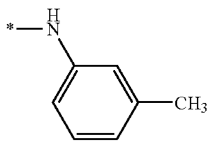
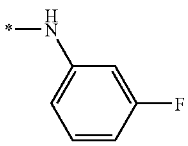
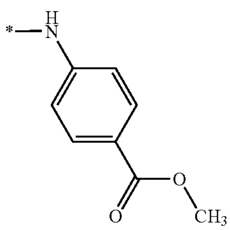
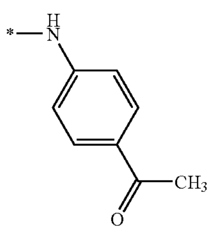
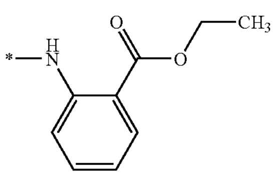
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
329		3.4	512	90
330		3.95	550	90
331		3.76	510	90
332		3.58	526	90
333		4.04	582	90

EXAMPLE 19

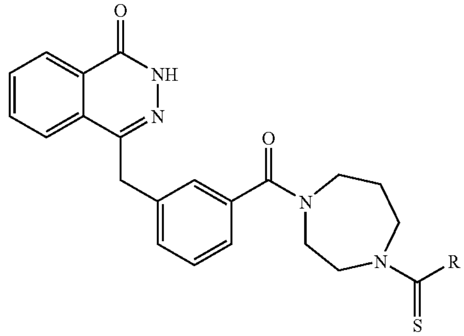
The appropriate isothiocyanate (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

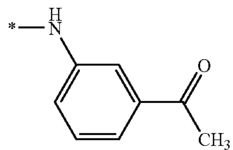
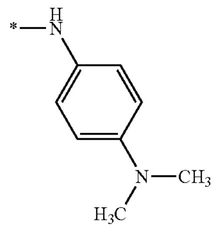
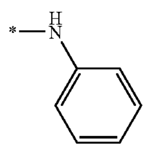
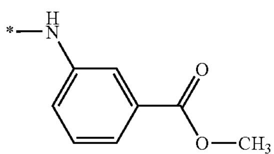
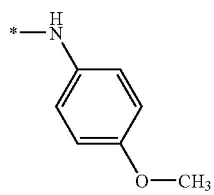
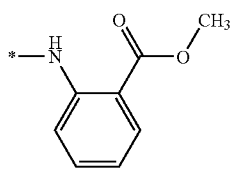
The compounds synthesised are set out below.



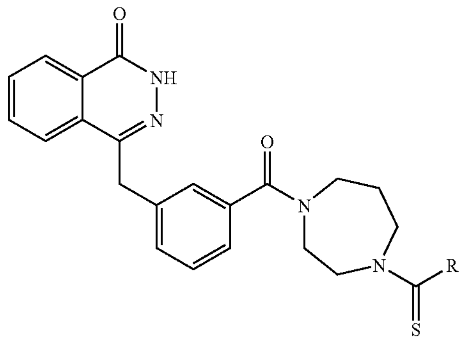
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
334		3.86	512	90
335		3.75	516	90
336		3.66	556	90
337		3.57	540	90
338		4.17	569	90

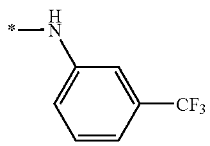
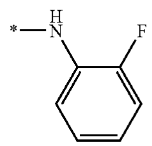
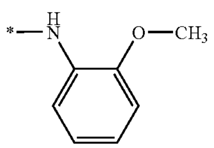
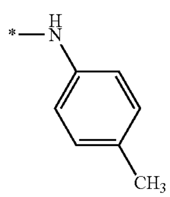
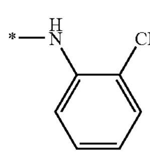
-continued



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
339		3.56	540	90
340		3.1	541	85
341		3.84	498	90
342		3.64	556	90
343		3.53	528	90
344		3.92	556	90

-continued

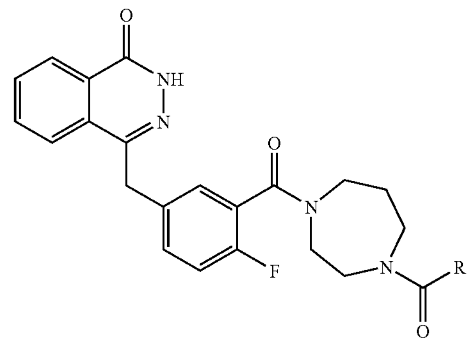


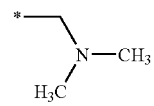
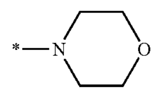
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
345		4.09	566	90
346		3.64	516	90
347		3.57	528	90
348		3.78	512	90
349		3.62	512	90

EXAMPLE 20

The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one (4) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

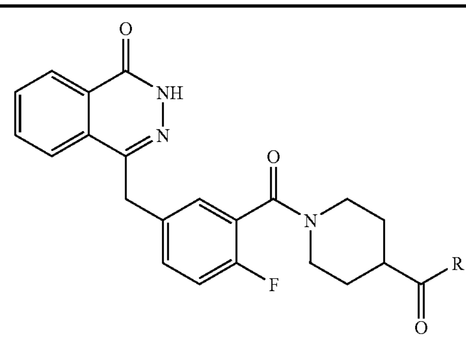


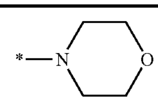
Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
372		2.81	466	80
385		3.19	495	90

EXAMPLE 21

1-[2-Fluoro-5-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]-piperidine-4-carboxylic acid (D) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M + 1	LC Purity (%)
386		3.21	480	90

EXAMPLE 22

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC_{50} values (Dillon, et al., *JBS.*, 8(3), 347-352 (2003)).

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25 mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50 mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 μM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 μl.

After 10 minutes incubation at 30° C. the reactions were initiated by the addition of a 10 μl reaction mixture, containing NAD (5 μM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30° C. for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 μl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded are counts per minute (cpm) following a 30 second counting of each well.

The % enzyme activity for each compound is then calculated using the following equation:

$$\%\ \text{Inhibition} = 100 - \left(100 \times \frac{(\text{cpm of unknowns} - \text{mean negative cpm})}{(\text{mean positive cpm} - \text{mean negative cpm})}\right)$$

IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited) were calculated, which are determined over a range of different concentrations, normally from 10 μM down to 0.001 μM. Such IC_{50} values are used as comparative values to identify increased compound potencies.

All compounds tested had a IC_{50} of less than 0.1 μM.

The following compounds have an IC_{50} of less than 0.01 μM: 2-5, 9, 10, 12-20, 24, 26-28, 30, 32-35, 38, 39, 42, 44-47, 49, 51, 58-68, 80-82, 84, 86, 88-90, 94-97, 100-105, 113-116, 121-123, 126, 132, 136, 138-140, 142-144, 147, 149, 151, 152, 156, 158, 159, 161-164, 166-175, 177-180, 182-184, 186-190, 194, 199, 202, 205, 207, 208, 213, 221-223, 225, 233-236, 239-274, 277, 279, 287, 288, 292, 293, 301-303, 306, 315, 316, 318-323, 325, 327-336, 338-350, 352-357, 359, 361, 363, 365 and 367-370.

The following compounds, as well as those above, have an IC_{50} of less than 0.02 μM: 1, 6-8, 11, 21-23, 25, 31, 36, 37, 40, 41, 43, 48, 52-54, 56, 57, 69-79, 83, 87, 91-93, 98, 99, 106, 109, 110, 111, 117, 118, 120, 124, 128-130, 133-135, 137, 141, 145, 146, 148, 150, 153-155, 157, 160, 165, 176, 181, 185, 191, 192, 195, 196, 197, 201, 203, 204, 206, 211, 212, 215-217, 219, 220, 224, 226, 227, 229-232, 237, 238, 275, 276, 278, 281-286, 289-291, 294, 295, 297-299, 304, 305, 307-309, 311, 314, 317, 324, 326, 337, 351, 358, 360, 362, 364, 366, 371 and 372.

The Potentiation Factor (PF_{50}) for compounds is calculated as a ratio of the IC_{50} of control cell growth divided by the IC_{50} of cell growth+PARP inhibitor. Growth inhibition curves for both control and compound treated cells are in the presence of the alkylating agent methyl methanesulfonate (MMS). The test compounds were used at a fixed concentration of 0.2 micromolar. The concentrations of MMS were over a range from 0 to 10 μg/ml.

Cell growth was assessed using the sulforhodamine B (SRB) assay (Skehan, P., et al., (1990) New calorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107-1112.). 2,000 HeLa cells were seeded into each well of a flat-bottomed 96-well microtiter plate in a volume of 100 μl and incubated for 6 hours at 37° C. Cells were either replaced with media alone or with media containing PARP inhibitor at a final concentration of 0.5, 1 or 5 μM. Cells were allowed to grow for a further 1 hour before the addition of MMS at a range of concentrations (typically 0, 1, 2, 3, 5, 7 and 10 μg/ml) to either untreated cells or PARP inhibitor treated cells. Cells treated with PARP inhibitor alone were used to assess the growth inhibition by the PARP inhibitor.

Cells were left for a further 16 hours before replacing the media and allowing the cells to grow for a further 72 hours at 37° C. The media was then removed and the cells fixed with 100 μl of ice cold 10% (w/v) trichloroacetic acid. The plates were incubated at 4° C. for 20 minutes and then washed four times with water. Each well of cells was then stained with 100 μl of 0.4% (w/v) SRB in 1% acetic acid for 20 minutes before washing four times with 1% acetic acid. Plates were then dried for 2 hours at room temperature. The dye from the stained cells was solubilized by the addition of 100 μl of 10 mM Tris Base into each well. Plates were gently shaken and left at room temperature for 30 minutes before measuring the optical density at 564 nM on a Microquant microtiter plate reader.

All the compounds tested had a PF_{50} at 200 nM of at least 2.0.

EXAMPLE 23

To assess the stand alone activity of a PARP inhibitor on Braca 1 and 2 deficient cells the following protocols were used.

Small Molecule Inhibitors of PARP:

Compound (4) was dissolved in DMSO at 10 mM and stored at −20° C. in the dark.

Cell Lines

VC8 cells and the mouse Brca2 BAC complemented derivatives were as described in M. Kraakman-van der Zwet, et al., *Mol Cell Biol* 22, 669-79 (2002)). ES cells defective in Brca2 function have been described previously (Tutt, et al., *EMBO Rep* 3, 255-60 (2002)). The construction of ES cells defective in Brca1 will be described elsewhere but have previously been validated (Foray, et al., *Embo J*, 22, 2860-71 (2003)).

Clonogenic Assays

For measurement of cellular sensitivity to a PARP inhibitor (compound 4), cell cultures in exponential growth were trypsinised and seeded at various densities in 6-well plates onto Mitomycin C inactivated mouse embryonic fibroblasts and where appropriate treated with the test compound after 18 hours. For continuous exposure, cells were re-fed every 4 days with fresh medium and inhibitor. After 10-14 days, cells were washed with PBS, fixed in methanol and stained with crystal violet. Colonies containing greater than approximately 50 cells were counted. Experiments were performed at least three times in triplicate.

Results

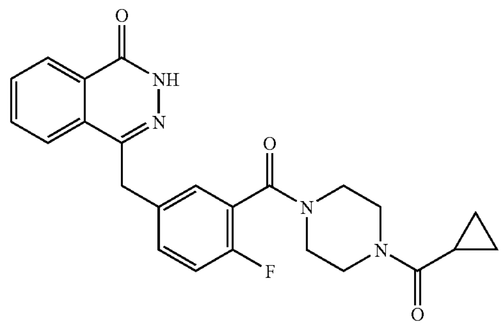
Reduction in the viability of BRCA1 and BRCA2 deficient cells Compound 4 was used to probe the sensitivity of cells deficient in Brca1 or Brca2 to the inhibition of PARP activity. Clonogenic assays showed that both Brca1 and Brca2 deficient cell lines were extremely sensitive to compound 4 compared to otherwise isogenic cells (FIG. **1**A, **1**B). The SF_{50} (dosage at which 50% of cells survived) for Compound 4 was 1.5×10^{-8}M for cells deficient in Brca1, whilst the SF_{50} for matched wild type cells was 7×10^{-6}M (FIG. **1**A). This represents a factor of 467 fold enhanced sensitivity of Brca1 mutant cells compared to wild type cells.

The SF_{50} for Compound 4 was 1.2×10-8M for cells deficient in Brca2 whilst the SF_{50} for matched wild type cells was 1.8×10^{-5}M (FIG. **1**B). This represents a factor of 1,500 fold enhanced sensitivity of Brca2 mutant cells compared to wild type cells. Similar results were obtained with Chinese hamster ovary cells deficient in Brca2 (VC8) compared to a Brca2-complemented derivative (VC8-BAC)(FIG. **2**). The SF_{50} for Compound 4 was 5×10^{-8}M for the Brca2 deficient VC8 line whilst the SF_{50} for matched control, VC8-BAC, was 3×10^{-5}M (FIG. **2**). This represents a factor of 600 fold enhanced sensitivity of Brca2 mutant cells compared to wild type cells.

The invention claimed is:

1. A compound of formula (III):

(III)



or isomers, salts, or solvates thereof.

2. A pharmaceutical composition comprising a compound according to claim **1** and one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, or stabilisers.

* * * * *